CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208573Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Venclexta (venetoclax)

Date: March 24, 2016
To: File for NDA 208573
From: John K. Leighton, PhD, DABT
Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting and labeling reviews for Venclexta conducted by Drs. Gudi and Place, and secondary memorandum and labeling provided by Dr. Sheth. In their Executive Summary, Drs. Gudi and Place discuss metabolite M27, present in humans at nearly 30% of total drug but representing less than 1% of drug in mice, rabbits and dogs. Additional general toxicology studies are not needed for this metabolite, consistent with the ICH S9 Guidance for Industry: Nonclinical Evaluation for Anticancer Pharmaceuticals. As the reproductive toxicology evaluation of venetoclax also demonstrated a potential risk to embryo-fetal development, additional reproductive toxicology studies are not needed to address the reproductive toxicity of M27. If the Applicant seeks other indications for this drug for which carcinogenicity studies are needed, the metabolite M27 should be adequately represented.

I concur with Dr. Sheth's conclusion that Venclexta may be approved for the proposed indication.

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/s/

JOHN K LEIGHTON
03/24/2016

MEMORANDUM

Date: March 21, 2016
From: Christopher Sheth, PhD
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
Re: Approvability for Pharmacology and Toxicology
NDA: 208573
Drug: Venclexta (venetoclax)
Indication: Treatment of patients with chronic lymphocytic leukemia (CLL) with 17p deletion, as detected by an FDA approved test, who have received at least one prior therapy.
Applicant: Abbvie, Inc.

Venetoclax is a selective and orally bioavailable small molecule inhibitor of the B-cell lymphocyte-2 (Bcl-2) anti-apoptotic protein, being developed for the above indication. Venclexta tablets will be initially taken at 20 mg once daily for 7 days, followed by a weekly ramp-up dosing schedule to the recommended daily dose of 400 mg. The Established Pharmacological Class of "Bcl-2 inhibitor" was determined to be both scientifically valid and clinically meaningful.

The pharmacology and toxicology studies reviewed included pharmacodynamics, safety pharmacology, genotoxicity, repeat dose toxicity, and reproductive and developmental toxicity. In vitro, venetoclax is cytotoxic to cells overexpressing Bcl-2 (a hallmark of various hematologic malignancies). Pharmacology studies indicate venetoclax-mediated apoptosis involves binding to Bcl-2, displacing pro-apoptotic proteins like BIM, triggering mitochondrial outer membrane permeabilization and the activation of caspases. Venetoclax was shown to have antitumor activity in vivo in a human acute lymphocytic leukemia xenograft (mouse) model. Additionally, lymphocyte decreases were observed in the toxicology studies, which is an expected effect of Bcl-2 inhibition.

The potential for adverse venetoclax-mediated effects on the central nervous system and respiratory systems was evaluated in rodents, and cardiovascular safety pharmacology endpoints were as evaluated in an in vitro hERG assay and in vivo in dogs. Venetoclax has an acceptable safety pharmacology profile at doses achieving plasma concentrations relevant to the recommended human daily dose of 400 mg. Repeat dose general toxicology studies (26-week mouse and 39-week dog) indicate that venetoclax primarily affects the hematologic system (decreased lymphocytes and red blood cell mass), and the male dog reproductive system (testicular germ cell depletion in dogs).

Based on the findings in animals, Venclexta may compromise male fertility and cause fetal harm. Fertility and early embryonic development studies in male and female mice revealed no effects of venetoclax on estrus cycles, mating, fertility, corpora lutea, uterine implants or live embryos per litter at doses up to 600 mg/kg/day, which is approximately 2.8 and 3.2 times the human exposure at 400 mg (based on AUC) in male and female mice, respectively. No teratogenicity was observed in either the mouse or the rabbit embryofetal development studies. Venetoclax

was fetotoxic in mice at 150 mg/kg/day (a dose yielding exposures approximately 1.2 times the human exposure at 400 mg (based on AUC), and was also associated with post-implantation loss and decreased fetal body weights. In rabbits, venetoclax produced maternal toxicity at 300 mg/kg/day, but no fetal toxicity (300 mg/kg/day approximates 0.2 times the human exposure at 400 mg (based on AUC). The label will state that woman should discontinue breastfeeding while taking Venclexta.

No carcinogenicity studies have been conducted with venetoclax. Venetoclax was not mutagenic in a bacterial mutagenicity (Ames) assay, did not induce numerical or structural aberrations in an in vitro assay using human peripheral blood lymphocytes, and was not clastogenic in an in vivo mouse bone marrow micronucleus assay.

The nonclinical studies needed to support product labeling were reviewed by Drs. Ramadevi Gudi and Emily Place. The nonclinical findings are summarized in the "Executive Summary" of the NDA review and reflected in the product label.

Recommendation: I concur with the pharmacology/toxicology reviewers that from a nonclinical perspective, Venclexta may be approved and that no additional nonclinical studies are needed to support approval of Venclexta for the proposed indication.

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/s/

CHRISTOPHER M SHETH
03/21/2016

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	NDA 208573
Supporting document/s:	1
Applicant's letter date:	September 15, 2015
CDER stamp date:	September 15, 2015
Product:	Venetoclax, ABT-199 (A-1195425)
Indication:	Patients with relapsed or refractory chronic lymphocytic leukemia who have received at least one prior therapy, including those with 17p deletion.
Applicant:	Abbvie, Inc.
Review Division:	Division of Hematology Oncology Toxicology (DHOT)
Reviewers:	Ramadevi Gudi, PhD Emily Place, PhD, MPH
Supervisor/Team Leader:	Christopher M. Sheth, PhD
Division Director:	John Leighton, PhD, DABT
Project Manager:	Beatrice Kallungal, BS

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 208573 are owned by Abbvie, Inc. or are data for which Abbvie, Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 208573 that Abbvie, Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 208573.

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1 Executive Summary

1.1 Introduction

Venetoclax (Venclexta) is an orally administered B-cell lymphocyte-2 (Bcl-2) inhibitor that restores programmed cell death in cancer cells. Venclexta is indicated for the treatment of patients with relapsed or refractory chronic lymphocytic leukemia (CLL) who have received at least one prior therapy, including those with 17p deletion. Based on clinical trials submitted to support the use of venclexta in the intended patient population, the recommended dose is 400 mg daily.

1.2 Brief Discussion of Nonclinical Findings

Nonclinical pharmacology studies conducted in vitro and in vivo demonstrated that venetoclax inhibits Bcl-2, an anti-apoptotic protein regulator. In a series of biochemical assays conducted to characterize binding affinity, venetoclax demonstrated selectivity to Bcl-2 (Ki <0.1 nM) relative to other anti-apoptotic and pro-apoptotic complexes. In vitro studies using knockout (Bak-/- Bax-/-) murine embryonic fibroblasts demonstrated that venetoclax-mediated cell death requires the key effector proteins Bax and/or Bak, indicating activation of intrinsic pathway of apoptosis. Venetoclax showed selectivity in cell killing in tumor cells dependent on Bcl-2 for survival relative to tumor cells dependent on other anti-apoptotic family members (Bcl-XL cells). Increased sensitivity to venetoclax-mediated cell death was observed in leukemia and lymphoma cells harboring the t(14;18) translocation that overexpress Bcl-2. Venetoclax-induced apoptotic cell death was associated with release of mitochondrial intermembrane protein cytochrome C, caspase activation and the externalization of phosphatidylserine at the cell membrane. Venetoclax promotes cell death in a variety of hematological tumor cell lines including CLL cells derived from patients with an average EC_{50} of 6 nM (n=35). Venetoclax induced cell death in CLL samples bearing the 17p deletion derived from patients, with an average EC_{50} of 8 nM (n = 5). Additionally in SCID (Severe Combined Immunodeficiency) mice models of human xenografts expressing high levels of Bcl-2, treatment with venetoclax resulted in reduction of tumor volume.

Venetoclax treatment had no toxicologically significant effects on safety pharmacology endpoints including those from mouse (respiratory or neurological) and dog (cardiovascular) studies.

After oral dosing, bioavailability of venetoclax was 27% in the mouse and 28% in the dog. Tissue distribution was extensive following administration of oral venetoclax in rats. The highest exposure (by C_{max} or AUC) was in the liver, lymph nodes, small intestine, adrenal glands, kidney cortex, kidney, and the pancreas. The metabolism of venetoclax was similar in both mice and dogs following oral exposure; the metabolites in both mouse and dog plasma account for less than 13% of total drug related materials. The most prominent human metabolite “M27” was detected in both species (0.21% in dogs, 0.79% in mouse). The main route of elimination is the hepatobiliary system. Approximately 90% of elimination occurred in the feces, and less than 1% in the urine in

both mouse and dog. The elimination half-life after oral dosing in nonclinical species ranged from 3 to 14.5 hours. Based on the data collected in general toxicology studies, there were no gender differences in exposure, and increase in C_{max} and AUC values were dose proportional.

The toxicity of repeated daily doses of oral venetoclax was assessed by conducting 26-week (6-month) and 39-week (9-month) toxicity studies in mice and dogs, respectively. In both mice and dogs, the major target organs of venetoclax toxicity included the lymphatic system, and male reproductive organs (dogs). Toxicities in mice and/or dogs included:

- Dose-related body weight reductions (up to 15%) correlated with decreased food consumption in dogs.
- Dose-responsive decrease in lymphocyte (up to -75% in mice, and -81% in dogs) and red blood cell mass decreases in mice and dogs. Decrease in lymphocytes correlated with microscopic findings in lymphoid organs including the mandibular and mesenteric lymph nodes, and spleen, and gut-associated lymphoid tissue (GALT).
- Male reproductive systems (decreased prostate weights, dose dependent bilateral testicular seminiferous tubule degeneration/atrophy, reduced testicular weight) in dogs.
- Epithelial single cell necrosis (gallbladder, exocrine pancreas, prostate, epididymides, and stomach) in dogs.
- Hair discoloration correlated microscopically with decreased pigment in the hair follicle bulbs in the scapular region in dogs.

Venetoclax was not phototoxic to hairless mice when administered orally daily for three days up to 825 mg/kg followed by UV exposure.

Venetoclax did not induce mutations in the bacterial mutagenesis (Ames) assay, and was not clastogenic in both the in vitro chromosome aberration assay using human peripheral blood lymphocytes and the in vivo bone marrow mouse micronucleus assay. Carcinogenicity studies have not been conducted and are not necessary for the proposed indication. M27 was negative in the Ames assay and in the vitro chromosome aberration studies.

In fertility and early embryonic development studies conducted in male and female mice, venetoclax had no effect on male fertility, or female fertility parameters (e.g. estrous cycling, mating, or early embryonic development).

The embryo-fetal development effects of venetoclax were studied in mice and rabbits. Venetoclax produced decreases in implantations, litter size, live fetuses, fetal body weights, increases in both dead or resorbed conceptuses/litter and number of post implantation losses in mice. These effects are supported by scientific literature

indicating the role of BCL-2 in oocyte and embryonic development. In addition, BCL-2 knockout mice exhibited adverse developmental effects, such as renal failure.[1], [2],[3] ,[4],[5]. Thus, administration of venetoclax during pregnancy may cause embryo-fetal toxicities and a statement under the Warnings and Precautions of the label is warranted. Venetoclax was not teratogenic in mice. Of note, the human metabolite M27, present at nearly 30% in patients at the recommended dose of 400 mg/day was present in minor amounts in the mouse (0.8%). In rabbits, venetoclax was maternally toxic based on mortality (4/20) and reductions in net body weight gain (57% of the control), most evident at the high dose. While no embryo-fetal effects were observed in rabbits, this species may not predict adverse embryo-fetal effects in humans because the exposure to the parent drug was very low (0.2 times the human exposure) and metabolite M27 is not present in rabbits.

The dose of 150 mg/kg/day of venetoclax in mice resulted in exposures (AUC) of approximately 1.2 times (AUC 37.8 µg*hr/mL in mice) and the dose of 300 mg/kg/day of venetoclax in rabbits resulted in exposures (AUC) of approximately 0.2 times (AUC 4.9 µg*hr/mL in rabbits), the human exposure (AUC 31.8 µg*hr/mL) at the recommended dose of 400 mg daily.

1.3 Recommendations

1.3.1 Approvability

From a Pharmacology/Toxicology perspective, the approval of venetoclax (Venclexta) is recommended.

1.3.2 Additional Non-Clinical Recommendations

None

1.3.3 Labeling

The recommendations to the Applicant's proposed labeling were discussed internally and communicated to the Applicant. Information in the nonclinical sections of the label reflects findings of studies reviewed within this document.

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[1] Boumela et., al., 2011. Review - Involvement of BCL2 family members in the regulation of human oocyte and early embryo survival and death: gene expression and beyond Reproduction 141, 549-561[2] Veis Novacket., al., 1994. Bcl-2 Protein Expression During Murine Development. American Journal ofPathology, Vol. 145, No. 1, July 1994

[3] LeBrun et., al., 1993. Expression of bcl-2 in Fetal Tissues Suggests a Role in Morphogenesis American Journal ofPathology, Vol. 142, No. 3

[4] Yuan Yang et., al., 2002. Expression of Bcl-2 and Bax proteins in relation to quality of bovine oocytes and embryos produced in vitro. Animal Reproduction Science Volume 70, 159–169

[5] Fedorov et., al., 2006. Renal failure causes early death of *bcl-2* deficient mice Mechanisms of Ageing and Development Volume 127, Pages 600–609

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number
1257044-40-8

2.1.2 Chemical Abstracts Service (CAS) Name
Benzamide, 4-[4-[[2-(4-chlorophenyl)-4,4-dimethyl-1-cyclohexen-1-yl]methyl]-1-piperazinyl]-*N*-[[3-nitro-4-[[(tetrahydro-2*H*-pyran-4-yl)methyl]amino]phenyl]sulfonyl]-2-(1*H*-pyrrolo[2,3-*b*]pyridine-5-yloxy)-

2.1.3 Generic Name
Venetoclax

2.1.4 Synonym(s)
A-1195425.0; ABT-199; GDC 0199

2.1.5 Chemical Name
4-(4-{[2-(4-chlorophenyl)-4,4-dimethylcyclohex-1-en-1-yl]methyl}piperazin-1-yl)-N-({3-nitro-4-[(tetrahydro-2H-pyran-4-ylmethyl)amino]phenyl}sulfonyl)-2-(1H-pyrrolo[2,3-b]pyridin-5-yloxy)benzamide

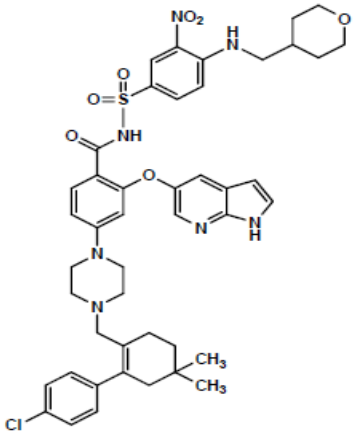
2.1.6 Molecular Formula
C45H50ClN7O7S

2.1.7 Molecular Weight
868.44

2.1.8 Structure

Figure 1: Structure or Biochemical Description of Venetoclax

(Excerpted from Applicant's NDA)



Pharmacologic Class
Bcl-2 inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND-110159 ABT-199 (venetoclax)

2.3 Drug Formulation

Venclexta tablets for oral administration are pale yellow or beige tablets that contain 10, 50, or 100 mg venetoclax as the active ingredient. The composition showing the amount of each component per tablet is presented in Table 1.

Table 1: Composition of Venclexta 10, 50, and 100 mg Tablets

(Excerpted from Applicant's NDA)

			Dosage Strength	10 mg	50 mg	100 mg
			List Number	000561	000566	000576
Component	Quality Standard	Function	Amount (mg)/Tablet			
Tablet Core						
(b) (4)						
Venetoclax	In-house standard	Drug Substance		10.0	50.0	100.0
Copovidone, (b) (4)	NF / Ph. Eur.	(b) (4)				
Polysorbate 80	NF / Ph. Eur.					
Colloidal Silicon Dioxide/ (b) (4)	NF / Ph. Eur.					
(b) (4)						
Anhydrous Dibasic Calcium Phosphate	USP / Ph. Eur.					
(b) (4)	NF/Ph. Eur.					
Sodium Stearyl Fumarate	NF/Ph. Eur.					
(b) (4)						

The physical appearance, marking, shape, color, coating and dimensions of the tablets are summarized in Table 2.

Table 2: Description of Venclexta Tablets

(Excerpted from Applicant's NDA)

	Dosage Strength		
Parameter	10 mg	50 mg	100 mg
Appearance	Tablet	Tablet	Tablet
Coating	Film coated	Film coated	Film coated
Shape	Round, biconvex	Oblong, biconvex	Oblong, biconvex
Color	Pale yellow	Beige	Pale yellow
Marking	"V" on Side 1 "10" on Side 2	"V" on Side 1 "50" on Side 2	"V" on Side 1 "100" on Side 2
Approximate Dimensions	6.0 mm diameter	14.0 x 8.0 mm	17.2 x 9.5 mm

2.4 Comments on Novel Excipient

The compendial excipients used in the manufacture of venetoclax tablets are listed in the following table.

Table 3: Compendial Excipients in the Drug Product

*(Excerpted from Applicant's NDA_*

Excipient	Quality Standard
Copovidone, (b) (4)	NF/Ph. Eur. [a]
Polysorbate 80	NF/Ph. Eur.
Colloidal Silicon Dioxide (b) (4)	NF/Ph. Eur.
Anhydrous Dibasic Calcium Phosphate	USP/Ph. Eur.
Sodium Stearyl Fumarate	NF/Ph. Eur.
(b) (4)	

Specification for Copovidone, (b) (4)

Copovidone, (b) (4) conforms to the requirements set forth in the National Formulary (NF) and the European Pharmacopoeia (Ph. Eur.).

2.5 Comments on Impurities/Degradants of Concern

For the purposes of the proposed oncology indication, all impurities were either qualified in nonclinical studies or within acceptable limits according to ICHQ3A and ICHQ3B.

2.6 Proposed Clinical Population and Dosing Regimen

Venetoclax is indicated to treat patients with chronic lymphocytic leukemia (CLL),including patients who have received at least one prior therapy (relapsed/refractory [R/R]) (b) (4) that have a 17p deletion (b) (4). The recommended dose and schedule of venetoclax is 400 mg, taken orally once per day.

2.7 Regulatory Background

Venetoclax (ABT-199, GDC-0199) was investigated under IND 110159 as a single agent treatment of either patients with relapsed or refractory (R/R) chronic lymphocytic leukemia (CLL) who specifically harbor the 17p deletion (17p del) mutation. Abbvie and Genentech/Roche (the Sponsors) are jointly developing venetoclax under IND 110159 and IND 115045 for the potential treatment of patients with hematologic malignancies. On February 27, 2015, the Sponsors requested breakthrough therapy designation and provided justification that venetoclax demonstrated substantial improvement over existing therapies in the treatment of patients with R/R chronic lymphocytic leukemia who harbor the17p deletion mutation (17p del R/R CLL). Subsequently, on April 27, 2015, the FDA granted breakthrough therapy designation to venetoclax for the treatment of patients with 17p del R/R CLL.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacology:

Study #	Study title
R&D/10/905	In Vitro Biochemical Characterization of ABT-199.
Memo-24	Binding Affinity and Cellular Activity of A-1616724 and A-1621332
R&D/10/1025	In Vitro Pharmacology of ABT-199 – Activity Against Patient-Derived Chronic Lymphocytic Leukemia Cells Ex Vivo
R&D/10/538	In Vitro Pharmacology of ABT-199 - Activity Against Patient-Derived Chronic Lymphocytic Leukemia Cells Ex Vivo (Additional Data)
R&D/10/889	Growth Inhibition of Xenografted Human Hematological Tumors by the Bcl-2 Selective Inhibitor, ABT-199
R&D/10/974	Determination of the Minimal Efficacious Dose of ABT-199 When Combined with Rituximab (Rituxan®) for the Treatment of Human SU-DHL-4 NHL Tumors in C.B-17 SCID-BEIGE Mice
R&D/10/975	Determination of ABT-199 Antitumor Efficacy in the Human Toledo NHL Xenograft Model in C.B-17 SCID-BEIGE Mice

Secondary Pharmacology:

Study #	Study title
R&D/10/833 (b) (4)	In Vitro Pharmacology: (b) (4)
R&D/10/834 (b) (4)	In Vitro Pharmacology: Binding Assays
R&D 150024- (b) (4)	In Vitro Pharmacology
R&D 150024- (b) (4)	In Vitro Pharmacology
R&D 141267- (b) (4)	In Vitro Pharmacology

Safety Pharmacology:

Study #	Study title
TD10-040	A Neurobehavioral Safety Evaluation of Orally Administered A-1195425 (ABT-199) in Mice
TD10-041	A Respiratory Safety Evaluation of Orally Administered A-1195425 (ABT-199) in Mice
TD10-042	Study title: A cardiovascular Safety Evaluation of Orally Administered A-1195425(ABT-199) in Beagle Dogs
R&D/10/795	A-1195425: In Vitro Effects on hERG Current

PK/ADME

Study #	Study title
Memo No. 3	A-1195425 Pharmacokinetics following Intravenous or Oral Dosing in Mouse
Memo No. 6	A-1195425 Pharmacokinetics following Intravenous or Oral Dosing in Dog
R&D/13/052	Quantitative Whole-Body Autoradiography of Pigmented Rats Following Oral Administration of 14C-ABT-199
Memo 16	Preliminary Metabolite Identification of A-1195425 (ABT-199) in Plasma Samples from Mouse, Dog and Human
Memo No. 23	Plasma Concentrations of M27 following Oral Administration of A-1195425 in Mouse and Dog
R&D/14/0704	Absorption, Distribution, Metabolism and Excretion of [3H]A-1195425 in Male CD-1 Mice
R&D/14/0705	Absorption, Distribution, Metabolism and Excretion of [14C]A-1195425 in Male Beagle Dogs

Repeat-dose Toxicology

Study #	Study title

R&D/12/522	26-Week Oral Gavage Toxicity Study Of A-1195425 (b) (4) in CD-1 Mice
R&D/12/384	39-Week (Oral (b) (4) Dose) Toxicity Study of A-1195425 (b) (4) In Beagle Dogs

Genetic Toxicology

Study #	Study title
R&D/10/420	Salmonella-Escherichia coli Mammalian- Microsome Reverse Mutation Assay with a Confirmatory Assay with A-1195425.
R&D/10/421	Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes With A-1195425
R&D/12/675	In Vivo Mouse Bone Marrow Micronucleus Assay with A-1195425 (b) (4)
R&D/14/1122	Bacterial Reverse Mutation Assay in 6-Well Plates with A-1621332 (b) (4)
R&D/14/1123	In Vitro Mammalian Chromosome Aberration Assay in Human Peripheral Blood Lymphocytes (HPBL) with A-1621332 (b) (4)
R&D/13/545	Four-Week Oral (Gavage) Toxicity Study of A-1195425 (with Impurities) in CD-1 Mice

Reproductive and Developmental Toxicology

Study #	Study title
R&D/12/810	Study of Fertility and Early Embryonic Development to Implantation of A-1195425 (b) (4) Administered by Oral Gavage in Male Mice
R&D/13/279	Study of Fertility and Early Embryonic Development to Implantation of A-1195425 (b) (4) Administered by Oral Gavage in Female Mice
R&D/12/746	An Embryo-fetal Development Study of A-1195425 (b) (4) by Oral Gavage in Mice
R&D/12/824	An Embryo-fetal Development Study of A-1195425 (b) (4) by Oral Gavage in Rabbits

Other Toxicology Studies

Study #	Study title
R&D/15/0036	Combined Mammalian Erythrocyte Micronucleus Test of (b) (4) in Rat Bone Marrow and Comet Assay in Rat Liver
R&D/13/1119	Salmonella-Escherichia coli/Mammalian- Microsome Reverse Mutation Assay with (b) (4) and (b) (4)
R&D/14/0515	Bacterial Reverse Mutation Assay of (b) (4)
R&D/14/0516	In Vitro Mammalian Chromosome Aberration Assay in Human Peripheral Blood Lymphocytes (HPBL) with (b) (4)
R&D/14/0517	Bacterial Reverse Mutation Assay of (b) (4)
R&D/14/1110	In Vitro Mammalian Chromosome Aberration Assay in Human Peripheral Blood Lymphocytes (HPBL) with (b) (4)
R&D/14/0400	Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with (b) (4)
R&D/14/0401	Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes with (b) (4)
R&D/14/0518	(b) (4) Bacterial Reverse Mutation Test in Salmonella typhimurium and Escherichia coli
R&D/14/0519	(b) (4) In Vitro Mammalian Chromosome Aberration Test in Human Peripheral Blood Lymphocytes
R&D/15/0164	6-Well Bacterial Reverse Mutation Assay with (b) (4) of ABT-199
R&D/15/0241	6-Well Bacterial Reverse Mutation Assay of (b) (4) ABT-199
R&D/15/0326	6-Well Bacterial Reverse Mutation Assay with (b) (4) of ABT-199
R&D/15/0471	6-Well Bacterial Reverse Mutation Assay with (b) (4) ABT-199

Special Toxicology Studies

Study #	Study title
R&D/14/1173	Multiple Dose Oral Phototoxicity Evaluation of A-1195425 (b) (4) in Hairless Female Mice

3.2 Studies Not Reviewed

Primary Pharmacology:

Study #	Study title
R&D/10/976	Determination of the Minimal Efficacious Dose of ABT-199 (b) (4) in C.B-17 SCID-BEIGE Mice
R&D/12/401	Efficacy of ABT-199 in the Mouse Spontaneous NZBWF1 Model (b) (4)
R&D/12/507	ABT-199 Lymphocyte Phenotyping and Recovery Study in CD-1 Male Mice Following 4 Weeks of Oral Dosing
R&D/15/0782	Potential Mechanisms of Resistance to BCL-2-Selective Inhibitor ABT-199 (Venetoclax) Identified Using Cancer Cell Lines
R&D/13/316	BCL-2-Selective Inhibitor ABT-199 in (b) (4) and Patient Samples

Secondary Pharmacology:

Study #	Study title
R&D/10/835 (b) (4)	In Vitro Pharmacology
R&D/150007 (b) (4)	In Vitro Pharmacology
R&D 150024- (b) (4)	In Vitro Pharmacology
R&D 150122- (b) (4)	In Vitro Pharmacology
R&D 150123- (b) (4)	In Vitro Pharmacology

Safety Pharmacology:

Study #	Study title
R&D/09/1365	CNS/Neurobehavioral Safety Pharmacology Profile in the Rat (P.O. Administration)
R&D/10/766	Effects of A-1195425 on Cardiovascular and Hemodynamic Function in the Anesthetized Dog

PK/ADME

Study #	Study title
Drug Metabolism Memo # 04	A-1195425 Pharmacokinetics following Intravenous or Oral Dosing in Rats
Drug Metabolism Memo 05	A-1195425 Pharmacokinetics following Intravenous or Oral Dosing in Monkey
Drug Metabolism Memo No. 09	The In Vitro Permeability and Transport Characteristics of A-1195425 across Human Caco-2 Cells
Drug Metabolism Memo No. 18	A-1195425 Pharmacokinetics following Oral Administration in Rabbits
R&D/10/855	Absorption, Distribution, Metabolism and Excretion of [3H]A-1195425 in Male Sprague-Dawley Rats
R&D/10/857	Preclinical Pharmacokinetic Summary of A-1195425 Single Dose Studies in Mouse, Rat, Rabbit, Dog and Monkey
R&D/14/0500	Integration of Pharmaceutics, Formulations and Pharmacokinetics for the Definition of Maximum Feasible Exposures in Preclinical Studies with A-1195425
R&D/14/0875	Absorption, Distribution, Metabolism and Excretion (ADME) study of

	[14C]ABT-199 in Healthy Female Subjects of Non-Childbearing Potential Following a Single Oral Dose Administration
Drug Metabolism Memo No. 10	Determination of the Blood-to-Plasma Concentration Ratios Following Incubations of A-1195425 in Mouse, Rat, Dog, Monkey and Human Whole Blood
Drug Metabolism Memo No. 30	Determination of the Blood-to-Plasma Concentration Ratios Following Incubations of A-1621332 (A-1195425 M27 Metabolite) in Mouse, Rat, Dog, Monkey and Human Whole Blood
Drug Metabolism Memo No. 32	A-1195425 Plasma and Blood Concentrations following a 200 mg/kg Oral Dose in TgHRAS Mice
R&D/14/0801	Determination of the Unbound Fraction of A-1195425 to Human and Animal Plasma, Human Liver Microsomes and Bovine Serum Albumin
R&D/14/0877	Determination of the Unbound Fraction of A-1621332 (A-1195425 M27 Metabolite) in Mouse, Rat, Dog, Monkey and Human Plasma
Memo No. 08	Structural Characterization of A-1195425 Metabolites M2, M4 and M18 by NMR Spectroscopy
Memo No. 12	Assessment of Enzymes Involved in the Metabolism of A-1195425 Using UGT Recombinant Proteins
Memo No. 20	In Vitro Biotransformation of A-1617595 (M5) to Generate M27
Memo No. 22	Plasma Concentrations of M27 following Oral Administration of A-1195425 in Human
Memo No. 25	Preliminary Metabolite Identification of A-1195425 (ABT-199) in Plasma Samples from Rabbit
Memo No. 39	Formation of Reduction Products of A-1195425 in Human Feces
R&D/10/862	In Vitro Biotransformation of [3H]A-1195425
R&D/14/0876	Metabolism and Disposition of [14C]ABT-199 (A-1195425) in Female Subjects After a Single 200 mg Oral Dose
R&D/14/0882	Determination of the Metabolic Stability of A-1195425 in Liver Microsomes and Hepatocytes across Species
R&D/14/1184	Determination of the Metabolic Stability of A-1621332 (A-1195425 M27 Metabolite) in Liver Microsomes and Hepatocytes across Species
R&D/14/1186	Assessment of Drug Metabolizing Enzymes Involved in the Metabolism of A-1621332 (A-1195425 M27 Metabolite) Using Human Recombinant Enzymes

Repeat-dose Toxicology

Study #	Study title
R&D/12/395	A-1195425 Single Dose Dog Study with Pharmacokinetics and Lymphocyte Phenotyping Protocol V11-1824 (TX11-144)
R&D/09/1105	Seven-Day Oral Dosage Range-Finding Toxicity Study of A-1195425 in Beagle Dogs
R&D/10/1053	14-Day Oral Dosing Study of A-1195425 in Male CD1 Mice Study CMET09-176
R&D/10/342	4-Week Oral Toxicity Study of A-1195425 (b) (4) in Mice with a 4-Week Recovery Period
R&D/14/0737	Four-Week Oral Toxicity Study of A-1195425 (b) (4) in CByB6F1-Tg(HRAS)2Jic Wild Type (Non-Transgenic Littermates) Mice
R&D/14/0303	One-Week Oral Dose Range-Finding Toxicity Study of A-1195425 (b) (4) in CByB6F1-Tg(HRAS)2Jic Wild Type (non-Transgenic Littermates) Mice
R&D/14/0737	Four-Week Oral Toxicity Study of A-1195425 (b) (4) in CByB6F1-Tg(HRAS)2Jic Wild Type (Non-Transgenic Littermates) Mice
R&D/14/0959	Thirteen-Week Oral Maximum Tolerated Dose Toxicity Study of A-1195425 (b) (4) in Sprague-Dawley Rats
R&D/14/0193	Two-Week (Oral) Dose Range-Finding Toxicity Study of A-1195425 (b) (4) in Sprague-Dawley Rats

Reproductive and Developmental Toxicology

Study #	Study title

R&D/12/551	A Dose Range-finding Embryo-fetal Development Study of A-1195425 by Oral Gavage in Mice
R&D/12/746	An Embryo-fetal Development Study of A-1195425 (b) (4) by Oral Gavage in Mice
R&D/12/746	An Embryo-fetal Development Study of A-1195425 (b) (4) by Oral Gavage in Mice

3.3 Previous Reviews Referenced

The Pharmacology/Toxicology and other discipline reviews for IND 110159.

4 Pharmacology

4.1 Primary Pharmacology

The primary pharmacology studies submitted by the Applicant are adequate, confirming the mechanism of action as described in the label and demonstrating the anti-tumor activity of venetoclax under the non-clinical conditions tested.

Study No. R&D/10/905: In Vitro Pharmacology of Venetoclax

TR-FRET (time-resolved fluorescence resonance energy transfer) competition binding assays were conducted to assess the binding affinity of venetoclax to anti-apoptotic proteins Bcl-2, Bcl-XL, Bcl-w, and Mcl-1.

Methods: In this assay, venetoclax diluted in DMSO and further diluted using assay buffer was transferred to a plate and mixed with Terbium-labeled anti-GST antibody, Bak probe, and GST-Bcl family protein (Bcl-2, Bcl-XL, Bcl-w, or Mcl-1). Plates were incubated at room temperature for three hours before being read on an Envision Plate Reader using a Lantha Screen protocol (Excitation wavelength: 340 nM, Emission wavelength: 495 nM or 520 nM). Samples with no GST-Bcl family protein are run as low signal controls.

Results: As summarized in the following table, venetoclax exhibited higher affinity binding to Bcl-2 (Ki <0.10 nM), compared to Bcl-XL and Bcl-w and showed essentially no binding to Mcl-1.

Table 4: Effect of Venetoclax on Bcl Family Members – Binding Affinity

(Excerpted from Applicant's NDA)

Test	Assay	Test Compound	Results
Target Affinity			
Bcl-2	TR-FRET	ABT-199	$K_i = < 0.10$ nM
Bcl-X_L	TR-FRET	ABT-199	$K_i = 48$ nM
Bcl-w	TR-FRET	ABT-199	$K_i = 245$ nM
Mcl-1	TR-FRET	ABT-199	$K_i = > 444$ nM

Memo No. 24: Binding Affinity and Cellular Activity of A-1616724 and A-1621332

The binding affinity and activity of the major metabolite M27 (synthetic sample A-1621332) and A-1616724 ((b) (4) mixture of enantiomers, and a racemic mixture of the M27 metabolite) in the presence and absence of human serum were assessed.

Methods: TR-FRET binding assays as described above were used to assess the binding affinity of A-1616724, A-1621332 and venetoclax for the anti-apoptotic proteins Bcl-2, Bcl-XL and Mcl-1.

Results: As shown in the following table, A-1616724 and A-1621332 exhibit single digit nanomolar affinity for Bcl-2 that is > 430-fold less and > 220-fold less than that for venetovlax, respectively. A-1616724 and A-1621332 were not effective in killing either the Bcl-2-dependent or Bcl-XL dependent tumor cell lines (concentration required for 50% effect [EC_{50}] > 10,000 nM).

Table 5: In Vitro Potencies and Ratios of Venetoclax, A-1626724 and A-1621332

(Excerpted from Applicant's NDA)

	Biochemical assay (K_i) (μM)*				Cell viability (EC_{50}) (μM)*	
Compound	Human Serum (%)	Bcl-2	Bcl-X_L	Mcl-1	RS4;11 cells	Molt-4 cells
ABT-199	0	< 0.00001	0.0094	>0.44	-	-
	10	0.0024	>0.42	>0.41	0.058	>9.9
A-1616724	0	0.0043	>0.59	>0.32	-	-
	10	0.60	>0.66	>0.44	>10	>10
A-1621332	0	0.0022	>0.56	>0.33	-	-
	10	0.14	>0.66	>0.44	>10	>10

* Values reported are averages from at least triplicate runs.

	Biochemical assay				Cell viability	
Compound	Human Serum (%)	Bcl-2	Bcl-X_L	Mcl-1	RS4;11 cells	Molt-4 cells
ABT-199/A-1616724	0	≥ 430	≥ 63	-	-	-
	10	250	-	-	> 172	> 1
ABT-199/A-1621332	0	≥ 220	≥ 60	-	-	-
	10	≥ 58	-	-	> 172	> 1

Study No. R&D/10/905: In Vitro Pharmacology of Venetoclax

To evaluate the selectivity of venetoclax, the activity of venetoclax was tested against murine FL5.12 cells engineered to be dependent on either Bcl-2 or Bcl-XL for survival upon interleukin-3 (IL-3) withdrawal.

Methods: Murine FL5.12 Cells: FL5.12-Bcl-2 and FL5.12-Bcl-XL cells were propagated in RPMI with beta-mercaptoethanol and 10 μg/mL recombinant mouse IL-3. Prior to compound treatment, FL5.12 cells were washed twice in 50 mL PBS, resuspended in culture medium lacking IL-3 at $1x10^6$ cells/mL and grown for 48 hours. Cells were then seeded into 96-well microtiter plates at 50,000 cells per well and treated with venetoclax in half-log dilutions. Cells were incubated with venetoclax for 24 hours and their viability was assessed using Cell TiterGlo reagent (Promega) according to the manufacturer's instructions.

Results: As summarized in the following table, venetoclax killed FL5.12-Bcl-2 cells (EC_{50} = 4 nM), and showed much weaker cell killing activity against FL5.12-Bcl-XL cells (EC_{50} = 261 nM), indicating that this compound is functionally selective for Bcl-2.

Table 6: Effect of Venetoclax on Bcl Family Members – Functional Selectivity

(Excerpted from Applicant's NDA)

Functional Selectivity			
FL5.12-Bcl-2	Cell Viability	ABT-199	EC_{50} = 0.004 µM
FL5.12-Bcl-X_L	Cell Viability	ABT-199	EC_{50} = 0.261 µM

Venetoclax was also assessed in cellular mammalian two-hybrid assays to determine its effectiveness (potency) at disrupting complexes between anti-apoptotic family members (Bcl-2, Bcl-XL, or Mcl-1) and BH3-only pro-apoptotic proteins (Bim, Bcl-XS, or Noxa, respectively).

Methods: Mammalian two-hybrid HeLa cells stably expressing a luciferase reporter were transfected with a plasmid expressing both the "bait" and "prey" fusion-proteins (GAL4DBD-Bim and VP16AD-Bcl-2, GAL4DBD-Bcl-XL and VP16AD-Bcl-XS, or GAL4DBD-Mcl-1 and VP16AD-Noxa, respectively). All cells were transferred to culture medium lacking penicillin/streptomycin and hygromycin before adding the plasmid-Lipofectamine 2000 solution. The cells were then incubated for 24 hours, after which time they were treated with venetoclax at 0.0001 to 3 µM. After 24 hours, luciferase activity was assessed using the ONE-Glo kit (Promega, Madison, WI).

Results: Venetoclax was effective in disrupted Bcl-2-Bim complexes (EC_{50} = 3 nM), but was much less effective against Bcl-XL-Bcl-XS (EC_{50} = 2.167 µM) or Mcl-1-Noxa (EC_{50} >3.0 µM) complexes, further demonstrating the selectivity of this compound.

Table 7: Potency of Venetoclax on Bcl Family Members

(Excerpted from Applicant's NDA)

Functional Activity			
Bcl-2-Bim Mammalian Two-Hybrid	M2H	ABT-199	IC_{50} = 0.003 µM
Bcl-X_L-Bcl-X_S Mammalian Two-Hybrid	M2H	ABT-199	IC_{50} = 2.167 µM
Mcl-1-Noxa Mammalian Two-Hybrid	M2H	ABT-199	IC_{50} = > 3.000 µM

The cell killing activity of venetoclax was studied in a variety of human cancer cells lines in vitro. The majority these cells were positive for the t(14;18) translocation and had high levels of Bcl-2 expression.

Methods: RS4;11 cells (associated with overexpression of Bcl-2) were seeded at 50,000 per well in 96-well microtiter plates and treated with venetoclax in RPMI medium (Invitrogen) containing 10% human serum (Sigma) for 48 hours at 37°C in 5% CO_2. Venetoclax was diluted in half-log steps starting at 1 µM and ending at 0.001 µM. All other leukemia and lymphoma cell lines were seeded at 15,000-20,000 cells per well in 96-well microtiter plates and treated with compound in Iscove's Modified Dulbecco's Medium (IMDM, Invitrogen) or RPMI (Invitrogen) supplemented with 10% human serum (Sigma) for 48 hours at 37°C in 5% CO^2. Effects on proliferation were determined using Cell TiterGlo reagent (Promega) according to the manufacturer's instructions. IC_{50} values were determined by non-linear regression analysis of the concentration response data.

As shown below, venetoclax was especially active against cell lines expressing high levels of Bcl-2. Venetoclax demonstrated cell killing activity against a variety of lymphoma and leukemia cell lines, including B-cell follicular lymphomas (FL), mantle cell lymphomas (MCL), diffuse large B-cell lymphomas (DLBCL), acute myeloid leukemias (AML), and acute lymphocytic leukemias (ALL).

Table 8: Activity of Venetoclax Against Human Tumor Cell Lines

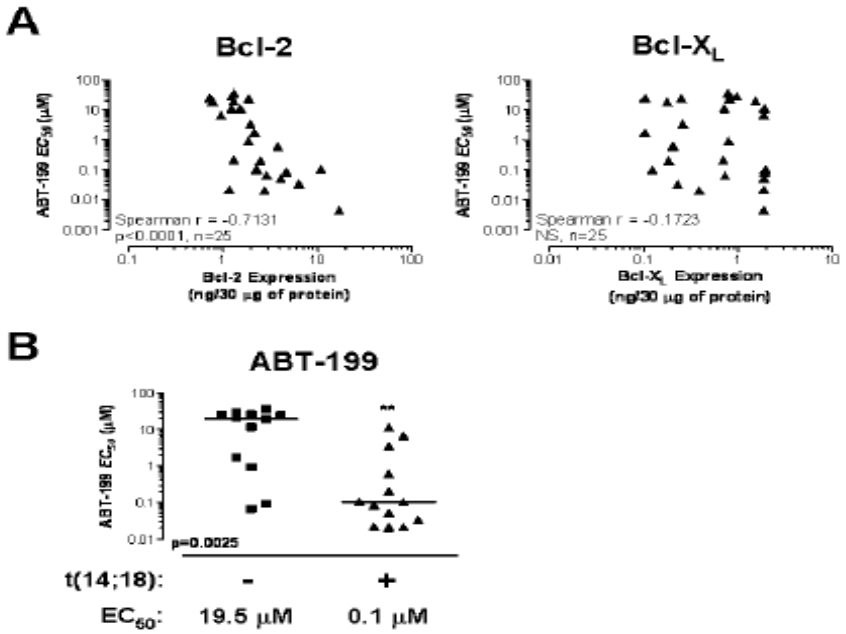
(Excerpted from Applicant's NDA)

Cell Line	Tumor Type	t(14;18) Translocation	ABT-199 (IC_{50}, µM)
DoHH-2	Follicular Lymphoma	yes	0.022
RS11380	Follicular Lymphoma	nd	0.046
Karpas-422	Follicular Lymphoma	yes	0.084
RL	Follicular Lymphoma	nd	0.086
Sc-1	Follicular Lymphoma	yes	0.340
Su-DHL-4	Follicular Lymphoma	yes	2.540
Mino	Mantle Cell Lymphoma	no	0.003
HBL2	Mantle Cell Lymphoma	no	0.004
GRANTA-519	Mantle Cell Lymphoma	no	0.031
U2932	DLBCL (ABC)	no	0.914
SU-DHL-2	DLBCL (ABC)	no	11.300
HBL1	DLBCL (ABC)	no	0.065
OCI-Ly3	DLBCL (ABC)	no	0.091
OCI-Ly1	DLBCL (GCB)	yes	0.004
OCI-Ly18	DLBCL (GCB)	yes	0.021
OCI-Ly19	DLBCL (GCB)	yes	0.103
SU-DHL-6	DLBCL (GCB)	yes	0.371
OCI-Ly8	DLBCL (GCB)	yes	0.610
OCI-Ly2	DLBCL (GCB)	no	1.715
WSU-DLCL2	DLBCL (GCB)	yes	6.607
WSU-NHL	DLBCL (GCB)	yes	11.266
OCI-Ly4	DLBCL (GCB)	no	18.780
HT	DLBCL (GCB)	no	23.900
OCI-Ly7	DLBCL (GCB)	no	24.280
SU-DHL-10	DLBCL (GCB)	no	24.910
U2940	DLBCL (PMBL)	no	20.130
Ki1106	DLBCL (PMBL)	no	27.920
MedB-1	DLBCL (PMBL)	no	36.103
Ri1	DLBCL (nd)	yes	0.004
NuDHL1	DLBCL (nd)	yes	0.052
MV4-11	AML	no	0.014
KG1a	AML	no	0.667
SEM	ALL	no	0.008
RS4;11	ALL	no	0.012

In vitro studies in leukemia and lymphoma cell lines demonstrate a stronger correlation between Bcl-2 expression and sensitivity to venetoclax than with Bcl-XL. Bcl-2 expressing cells that also harbor the t(14;18) translocation are exquisitely sensitive (Figure 2).

Figure 2: Expression Level of Bcl-2 – Inhibition of cell Proliferation by Venetoclax

(Excerpted from Applicant's NDA)



Mechanism of Action Studies

Assays were conducted to determine whether the mechanism of action of venetoclax includes the induction of the intrinsic apoptotic pathway.

The cell killing activity of venetoclax requires the pro-apoptotic effector proteins Bax and Bak, as demonstrated by studies with Bak-/- Bax-/- double knockout murine embryonic fibroblasts (MEFs). Bax and Bak are mediators of apoptosis and appear to be required for the initiation of the mitochondrial apoptosis pathway.[6],[7],[8]

Methods: Bax-/- Bak-/- knockout murine embryonic fibroblasts (MEFs) grown in DMEM supplemented with 10% FBS (Invitrogen) were seeded at 5,000 cells per well into 96-

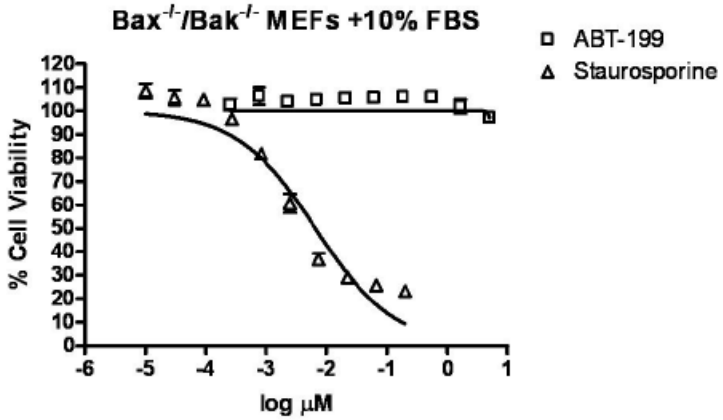
[6] Zong et., al., 2001. Proapoptotic BAX and BAK: A requisite gateway to mitochondrial dysfunction and death. Science. 2001;292:727-730.
[7] Lindsten et al., 2000. The combined functions of proapoptotic Bcl-2 family members Bak and Bax are essential for normal development of multiple tissues. Molecular Cell.6:1389-1399.
[8] Degenhardt 2002. Bax and Bak independently promote cytochrome c release from mitochondria. Journal of Biological Chemistry. 277:14127-14134.

well microtiter plates. They were then treated for 48 hours with either three-fold dilutions of venetoclax, starting at 5.0 µM and ending at 0.3 nM, or staurosporine, starting at 0.2 µM and ending at 0.01 nM. Cell viability was assessed using Cell TiterGlo reagent (Promega) according to the manufacturer's instructions.

As shown in the following figure venetoclax was ineffective against murine embryonic fibroblasts (MEFs) from transgenic mice lacking Bax and Bak.

Figure 3: Venetoclax - Inactivity Against Cells Lacking Bax and Bak

(Excerpted from Applicant's NDA)



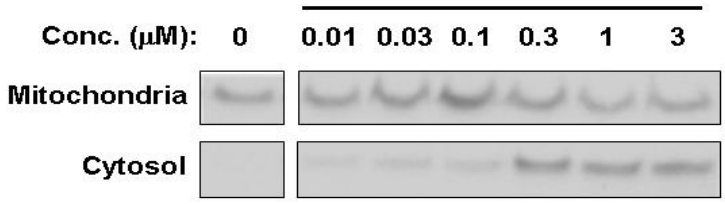
Cytochrome C Release Assay:

Methods: For each sample, $1x10^6$ RS4;11 cells were treated for four hours (37°C, 5% CO^2) with venetoclax (half-log dilutions starting at 3.0 µM and ending at 0.01 µM). Cells were then pelleted at 600 x g, washed , and resuspended in 100 µL permeabilization buffer (20 mM HEPES pH 7.2, 100 mM KCl, 5 mM MgCl2, 1 mM EDTA, 1 mM EGTA, 250 mM sucrose, 0.01% digitonin; all reagents from Sigma) by brief vortexing before incubating on ice for five minutes. Samples were spun down at 16,000 x g (4°C) for 10 minutes and the supernatants (cytosolic lysates) transferred to Eppendorf tubes on ice. Mitochondrial pellets were resuspended in 100 µL ONYX buffer (20 mM Tris pH 7.4, 135 mM NaCl, 1.5 mM MgCl2, 1 mM EGTA, 10% v/v glycerol, 1% Triton-X 100) supplemented with complete protease inhibitors, vortexed briefly, and spun down at 16,000 x g (4°C) for 10 minutes. The supernatants (mitochondrial lysates) were then transferred to Eppendorf tubes on ice. Equal volumes of each sample were boiled for 10 minutes in 1x LDS loading buffer (Invitrogen) and subjected to SDS-PAGE and immunoblotting. Blots were probed for cytochrome C using a mouse monoclonal antibody (clone 7H8.2C12, (b) (4)

Results: As shown in the figure below, a dose dependent increase in the release of cytochrome C was observed.

Figure 4: Venetoclax – Induction of Cytochrome C Release from Mitochondria

(Excerpted from Applicant's NDA)



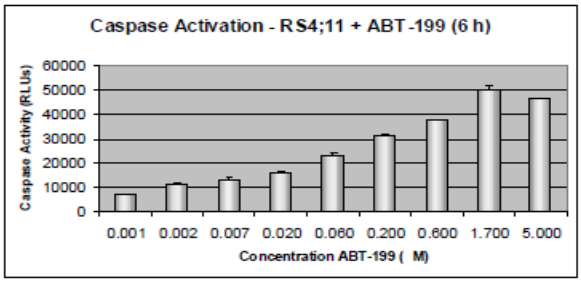
Caspase-3/-7 Activation Assay:

Methods: RS4;11 cells were seeded into 96-well plates at 5,000 cells per well in RPMI supplemented with 10% human serum (Sigma). Venetoclax in the same culture medium was added in half-log dilutions, starting at 5 µM and ending at 0.001 µM. The cells were then incubated at 37°C (5% CO_2) for 3.5 hours before being left at room temperature for another 0.5 hours. Caspase-3/7 activity was then assessed using the Caspase-GLO kit (Promega) according to the manufacturer's instructions.

Results: As shown in the figure below, a dose dependent increase in the caspase activation was observed.

Figure 5: Venetoclax – Induction of Caspase Activation

(Excerpted from Applicant's NDA)



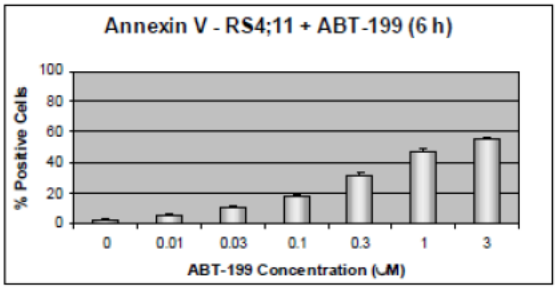
Release of phosphatidyl-serine Assay:

Methods: RS4;11 cells were seeded into 6-well tissue culture plates at $1x10^6$ per well in RPMI 1640 medium supplemented with 10% human serum . The cells were then treated for six hours with venetoclax in half-log dilutions starting at 0.01 to 3.0 µM . For each sample, $1x10^5$ cells were transferred to a well of a 96-well plate before adding 100 µL of Guava Nexin reagent . The samples were incubated at room temperature in the dark for 20 minutes before being analyzed on a Guava cell sorting apparatus according to the manufacturer's protocol.

Results: As shown in the figure below, a dose dependent increase in the release of phosphatidyl-serine by Annexin V staining was observed.

Figure 6: Venetoclax – Induction of Externalization of Phosphatidyl-Serine

(Excerpted from Applicant's NDA)



Study No. R&D/10/538 & R&D/10/1025: Potency of Venetoclax against CLL cells ex vivo

Venetoclax was assessed for its activity against patient-derived chronic lymphocytic leukemia (CLL) cells ex vivo.

Methods: CLL cells isolated from human subjects were treated with venetoclax (1-1000 nM) ex vivo for 24 hours. The viability of CD5+/CD19+ CLL cells was then determined for each sample by propidium iodide staining and flow cytometry. Venetoclax exhibited potent activity, killing CLL cells with an average IC50 of 6 nM (n=35).

Table 9: Activity of Venetoclax Against Patient-Derived CLL Cells

(Excerpted from Applicant's NDA)

Subject Number	Tumor Type	ABT-199 (IC_{50}, µM)
GS	CLL	(b) (4)
1107	CLL	
629	CLL	
631	CLL	
#50	CLL	
#87	CLL	
#90	CLL	
#91	CLL	
#92	CLL	
104	CLL	
106	CLL	
108	CLL	
110	CLL	
112	CLL	
113	CLL	

Table 10: Activity of Venetoclax Against Patient-Derived CLL Cells (Additional data)

(Excerpted from Applicant's NDA)

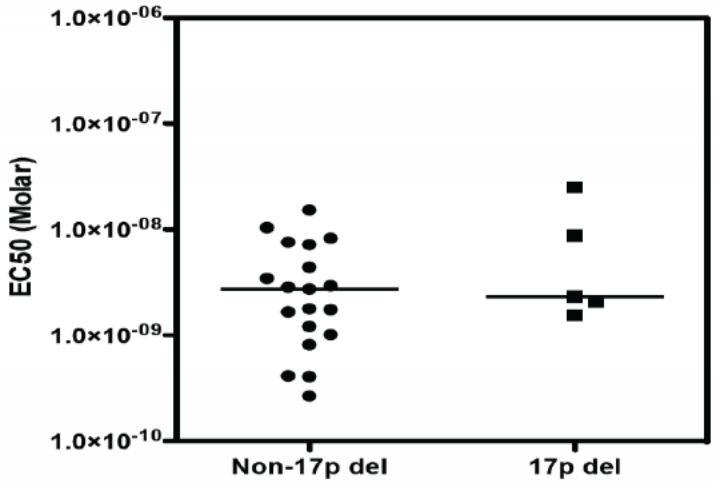
Table 1. Activity of ABT–199 Against Patient–Derived CLL Cells

Subject Number	Tumor Type	ABT-199 (IC_{50}, µM)
515	CLL	(b) (4)
516	CLL	
519	CLL	
523	CLL	
529	CLL	
24#2	CLL	
51	CLL	
52#3	CLL	
56	CLL	
57	CLL	
58	CLL	
59	CLL	
60	CLL	
65	CLL	
73	CLL	
606	CLL	
608	CLL	
610	CLL	
615	CLL	
617	CLL	

While the Pharmacology Written Summary stated that venetoclax was equally active against CLL samples bearing the 17p deletion with an average EC_{50} of 8 nM (n = 5), the study reports (Study No. R&D/10/538 & R&D/10/1025) did not identify the subjects bearing 17p deletion. An information request was sent to the Applicant on March 21, 2016. The Applicant will provide an addendum to report R&D/12/538 to include the information for the 5 samples with the 17p del mutation. Based on the response provided by the Applicant on March 21, 2016, the subject #s with 17p deletion were #91, 104, 106, 112 and 113 (Study No. R&D/10/538).

Figure 7: Activity of Venetoclax in CLL: Independent of 17p Deletion

(Excerpted from Applicant's NDA)



Activity studies of Venetoclax in Tumor Xenograft Models (R&D/10/889, R&D/10/974, R&D/10/975)

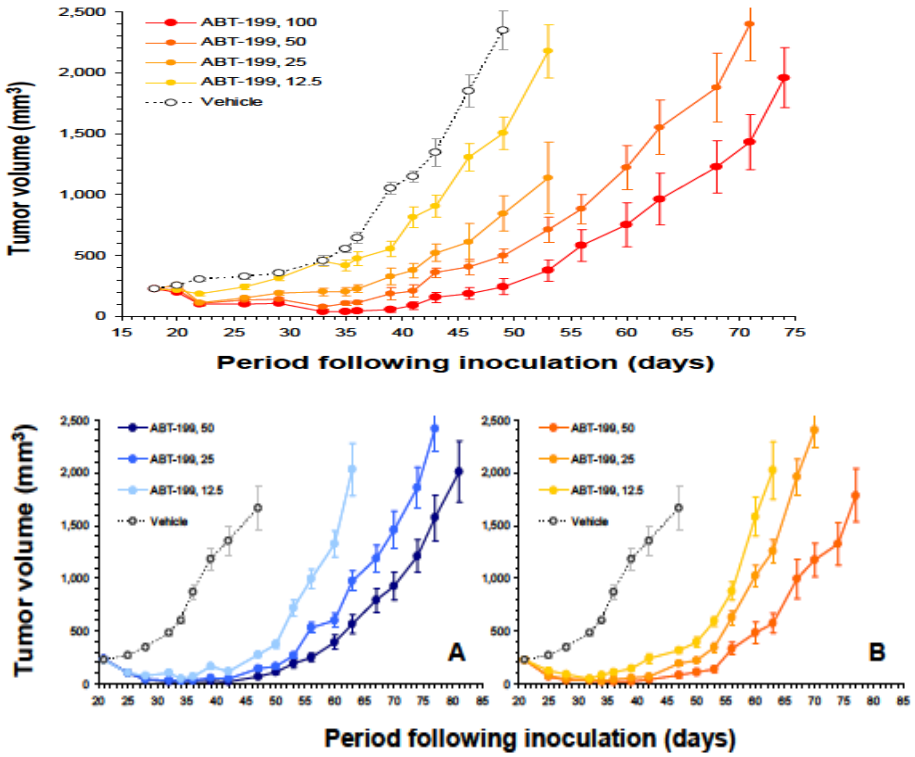
R&D/10/889: Activity studies of single or repeated administration of venetoclax were conducted in immunocompromised (severe combined immunodeficiency [SCID] or SCID-beige) mice bearing ALL, RS4;11 human tumor xenografts since ALL RS4;11 is associated with associated with overexpression of Bcl-2.

Methods: Venetoclax was administered to female SCID-bg mice as an oral formulation single oral dose at 0, 12.5, 25, 50 or 100 mg/kg/day on day 19, following tumor growth. Tumor sizes were matched on Day 18 and the mean tumor volume at staging was approximately 225mm^3. For repeated dose, venetoclax was administered to SCID-bg mice orally daily (QDx7) or twice a day (BIDx7) starting from day 22 after the tumors were size matched on Day 21. The mean tumor volume at tumor staging was approximately 230 mm^3).

Results: As shown in the following figures , venetoclax induced a reduction in tumor volume in 90% of animals (CR) in single or multiple dose regimens. A direct relationship between the dose and activity was also noted at this regimen.

Figure 8: Dose-Response Relationship of RS;11 Xenograft Growth Inhibition by Single Dose (top graph), Repetitive Daily (QDx7; A below) or Twice a Day (B below) Administration of Venetoclax

(Excerpted from Applicant's NDA)



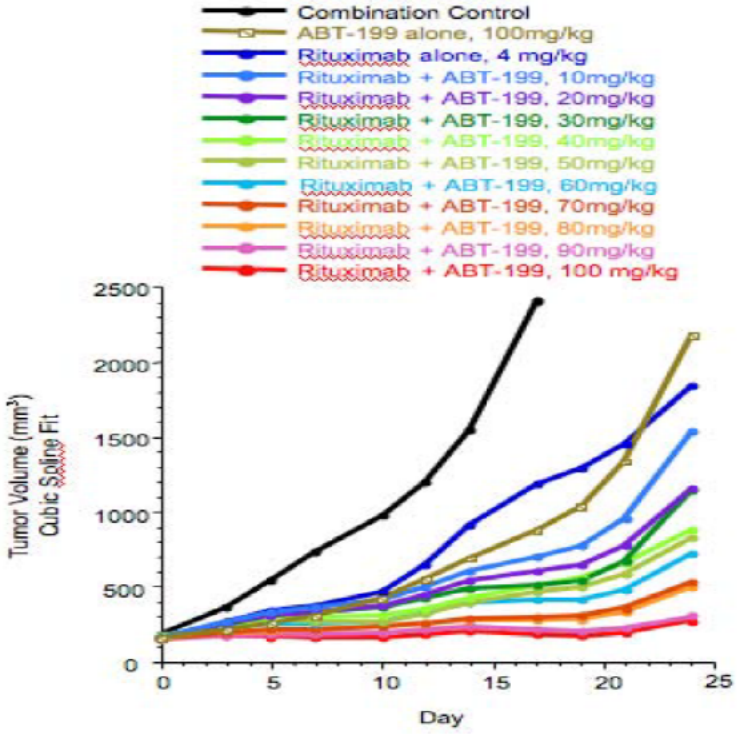
Study No. R&D/10/974: Determination of the Minimal Efficacious Dose of Venetoclax When Combined with Rituximab (Rituxan®) for the Treatment of Human SU-DHL-4 NHL Tumors in C.B-17 SCID-BEIGE Mice.

Methods: (SCID)-BEIGE mice bearing Human NHL SU-DHL-4 subcutaneous xenograft tumors (mean tumor volume at the time of group assignment was 162 mm^3) were administered with venetoclax or vehicle, QD for 21 days. Mice received control antibody (gD:5327) or rituximab on Days 1 and 8. Mice were dosed intravenously with rituximab 2 hours before oral administration of venetoclax when dosed in combination.

Results: As shown in the following figure, oral doses of venetoclax in combination with 4 mg/kg rituximab caused a dose-dependent increase in tumor growth inhibition of SU-DHL-4 xenografts. The % tumor growth inhibition (TGI) of human NHL SU-DHL-4 tumors observed with rituximab was 51% as well as in all venetoclax + rituximab combination groups versus the control (67%−99%). The maximal % TGI was 99% in the highest dose group (100 mg/kg venetoclax, 4 mg/kg rituximab). The minimal efficacious (activity) dose (ED_{50}) of venetoclax in combination with rituximab was 24 mg/kg.

Figure 9: Analysis of Venetoclax in Combination with Rituximab in the SU-DHL-4 NHL Xenograft Model

(Excerpted from Applicant's NDA)



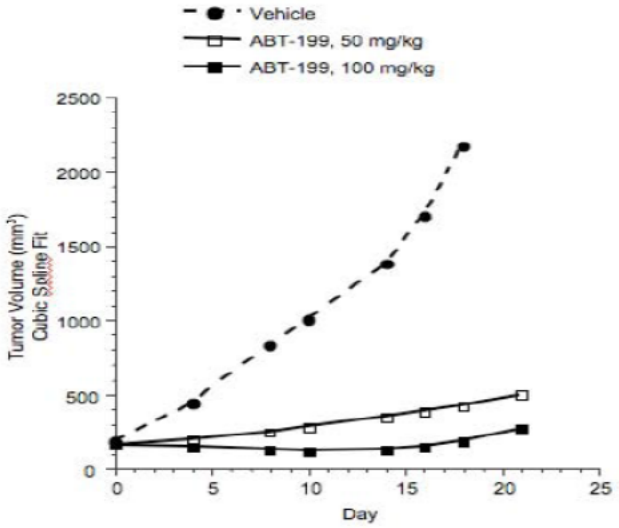
Note: Fitted tumor volumes after once daily oral administration of ABT-199 for 21 days (Days 1–21) with and without intravenous administration of rituximab at 4 mg/kg weekly for 2 weeks (Days 1 and 8). The dose of ABT-199 is expressed as (b) (4) equivalents in milligrams per kilogram body weight.

R&D/10/975: Determination of Venetoclax Antitumor Efficacy in the Human Toledo NHL Xenograft Model in C.B-17 SCID-BEIGE Mice

Methods: (SCID)-BEIGE mice bearing Human NHL Toledo subcutaneous xenograft tumors (mean tumor volume at the time of group assignment was 169 mm^3) were administered with venetoclax or vehicle, QD for 21 days.
Results: As shown in the following Table and figure, tumor growth inhibition of human NHL Toledo tumors was observed in animals dosed with both 50 and 100 mg/kg venetoclax (92% and 107%, respectively). Additionally, at the highest dose of venetoclax tested, 100 mg/kg, 30% of the mice demonstrated overall anti-tumor responses (as defined as the sum of partial and complete tumor responses).

Figure 10: Analysis of Venetoclax Tumor Growth Inhibition in the Toledo NHL Xenograft Model

(Excerpted from Applicant's NDA)



Note: Fitted tumor volumes after once daily oral administration of either ABT-199 or vehicle for 21 days (Days 1–21). The dose of ABT-199 is expressed as (b) (4) equivalents in milligrams per kilogram of body weight.

Overall, these data demonstrate that under the conditions studied, venetoclax induced cell killing in leukemia and lymphoma cells with specific chromosomal anomalies. CLL cells derived from patients harboring the 17p deletion we also sensitive to venetoclax, according to the Applicant's summary of the pharmacology data. Venetoclax inhibits subcutaneous xenograft growth of human tumor cell lines including, ALL (RS4;11) and NHL (Toledo).

4.2 Secondary Pharmacology

Study no: RD10833
Methods: Venetoclax was evaluated in an initial series of in vitro radioligand binding screening assays ((b) (4) receptor binding panel) that contained representatives of most G-protein coupled receptors and a set of ion channel binding sites. In each experiment data were compared with a reference standard and compared with in house historical controls. Venetoclax was tested at 10 μM in all assays.

Results: Venetoclax produced >50% displacement of control-specific binding in the following assays: adenosine-3 (A3), norepinephrine transporter, dopamine-5 (D5), PPARγ, prostacyclin (IP), peripheral benzodiazepine (BZD), and serotonin-5a (5-HT5a) receptors.

Study no: RD10834
Methods: In a follow up study, to determine conclusive binding of venetoclax to cellular and nuclear receptors using dose response assays. Binding was calculated as a % inhibition of the binding of a radiolabeled ligand specific for prostacyclin (IP), peripheral benzodiazepine (BZD), and serotonin-5a (5-HT5a), at concentrations of 0.1 to 10 μM (IC_{50} or EC_{50} determination). Inhibition or stimulation greater than 50% represents significant effects of venetoclax on receptor binding.

Results: Inhibition higher than 50% were observed for prostacyclin (Ki 0.81 μM), peripheral benzodiazepine (Ki 0.38 μM), and serotonin-5a (Ki 0.37 μM).

Study no: RD150024
Methods: In a follow up study, to determine conclusive binding of venetoclax to cellular and nuclear receptors using dose response assays. Binding was calculated as a % inhibition of the binding of a radiolabeled ligand specific for norepinephrine transporter, dopamine-5 (D5), PPARγ, at concentrations of 0.3 to 30 μM (IC_{50} or EC_{50} determination). Inhibition or stimulation greater than 50% represents significant effects of venetoclax on receptor binding.

Results: Inhibition or stimulation higher than 50% were not observed for norepinephrine transporter, dopamine-5 (D5), PPARγ.

Evaluation of M27

Study no: RD141267
Methods: Human metabolite M27 (A-1621332) was evaluated in an initial series of in vitro radioligand binding screening assays ((b) (4) receptor binding panel) that contained representatives of most G-protein coupled receptors and a set of ion channel binding sites. In each experiment data were compared with a reference standard and compared with in house historical controls. M27 was tested at 10 μM in all assays.

Results: At a concentration of 10 μM M27 produced >50% displacement of control-specific binding in the following assays: melatonin-2 (MT2, K_i >30 μM), estrogen (ERα), and delta-opioid (DOP) receptors.

Study no: RD150025
Methods: In a follow up study, to determine conclusive binding of M27 as a % inhibition of the binding of a radiolabeled ligand specific for melatonin-2 (MT2), estrogen (ERα), and delta-opioid (DOP), at concentrations of 0.3 to 30 μM (IC_{50} or EC_{50} determination). Inhibition or stimulation greater than 50% represents significant effects of M27 on receptor binding.

Results: Inhibition higher than 50% were observed only for the DOP receptor (K_i 0.65 μM, IC_{50} 1.1 μM).

4.3 Safety Pharmacology

Study title: **A Neurobehavioral Safety Evaluation of Orally Administered A-1195425 (ABT-199) in Mice**

Study no:	TD10-040 (R&D/10/315)
Study report location:	4.2.1.3
Conducting laboratory and location:	(b) (4) (b) (4).
Date of study initiation:	March 11, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1195425.0 (venetoclax) (b) (4) Lot# BREC-0190-085, 15.1 %w/w

Key study findings:
No venetoclax-induced neurobehavioral effects were noted up to 600 mg/kg, under the conditions tested.

Methods:

Doses:	50, 200, and 600 mg/kg/day
Frequency of dosing:	Single dose with neurobehavioral observations at 6 hours post-dose
Route of administration:	Oral gavage
Dose volume:	20 mL/kg
Formulation/Vehicle:	Vitamin E TPGS and Copovidone [23.5:76.5, %w/w]) in distilled water
Species/Strain:	Crl:CD1® (ICR) mice
Number/Sex/Group:	8 females
Age:	10 weeks
Weight:	25.0 to 28.6 grams
Satellite groups:	None

Mortality: All animals survived until termination of the study

Clinical Signs: unremarkable

Body Weights: unremarkable

Body temperature: unremarkable

Functional Observational Battery Evaluations: The observations included, evaluation of activity and arousal, posture, rearing, bizarre behavior, clonic and tonic movements, gait, mobility, stereotypy, righting reflex, response to stimulus (approach, click, tail pinch, and touch), palpebral closure, pupil response, piloerection, exophthalmus, lacrimation, salivation, and respiration. In addition, forelimb and hindlimb grip response, hindlimb splay, pain perception were also measured. No venetoclax-

related effects were noted for any of the neurobehavioral measures tested over the course of the study

Table 11: Summary of Functional Observational Battery - Predose, 4 Hour and 6 Hour Postdose

(Excerpted from Applicant's NDA)

Predose (Day -1)

Category	Observation	0 mg A-1195425.0/kg (Vehicle) Mean	SD	N	50 mg A-1195425.0/kg Mean	SD	N	200 mg A-1195425.0/kg Mean	SD	N	600 mg A-1195425.0/kg Mean	SD	N
Activity/Arousal	Rearing	25.6	9.12	8	33.0	9.65	8	21.8	11.32	8	26.8	7.29	8
Autonomic	Defecation	1.5	2.33	8	1.1	1.25	8	2.4	1.85	8	1.6	1.19	8
	Urination	0.1	0.35	8	0.6	1.77	8	0.0	0.00	8	0.0	0.00	8
Neuromuscular	Grip Response, sec	60.0	0.00	8	60.0	0.00	8	60.0	0.00	8	60.0	0.00	8
	Mean Hindlimb Splay, mm	41.83	5.252	8	37.62	6.224	8	36.38	2.156	8	41.75	5.108	8
Physiological	Body Temperature, °C	38.09	0.356	8	37.40[a]	0.475	8	37.66	0.833	8	38.19	0.181	8
	Body Weight, g	27.49	3.897	8	25.95	1.065	8	25.94	0.944	8	26.03	0.956	8
Sensorimotor	Thermal Response, sec	20.01	5.678	8	20.39	6.574	8	16.26	4.049	8	23.65	10.518	8

4 hour postdose

Category	Observation	0 mg A-1195425.0/kg (Vehicle) Mean	SD	N	50 mg A-1195425.0/kg Mean	SD	N	200 mg A-1195425.0/kg Mean	SD	N	600 mg A-1195425.0/kg Mean	SD	N
Activity/Arousal	Rearing	16.4	13.23	8	25.5	10.25	8	15.6	11.67	8	19.5	6.74	8
Autonomic	Defecation	3.5	1.69	8	3.9	1.46	8	2.3	1.75	8	3.5	0.76	8
	Urination	0.5	1.41	8	0.3	0.71	8	0.1	0.35	8	0.0	0.00	8
Neuromuscular	Grip Response, sec	60.0	0.00	8	60.0	0.00	8	60.0	0.00	8	60.0	0.00	8
	Mean Hindlimb Splay, mm	39.96	4.176	8	36.33	3.936	8	37.58	3.412	8	39.58	4.260	8
Physiological	Body Temperature, °C	37.98	0.480	8	37.96	0.329	8	37.89	0.458	8	38.11	0.517	8
	Body Weight, g	26.09	1.325	8	25.71	1.147	8	25.89	1.232	8	26.98	0.915	8
Sensorimotor	Thermal Response, sec	18.53	3.381	8	23.06	11.177	8	14.76	4.618	8	15.01	4.493	8

6 hour postdose

Category	Observation	0 mg A-1195425.0/kg (Vehicle) Mean	SD	N	50 mg A-1195425.0/kg Mean	SD	N	200 mg A-1195425.0/kg Mean	SD	N	600 mg A-1195425.0/kg Mean	SD	N
Activity/Arousal	Rearing	12.5	12.80	8	19.9	10.25	8	9.8	8.60	8	12.3	6.61	8
Autonomic	Defecation	1.9	1.36	8	2.0	2.14	8	2.5	1.93	8	2.1	1.89	8
	Urination	0.0	0.00	8	0.1	0.35	8	0.1	0.35	8	0.1	0.35	8
Neuromuscular	Grip Response, sec	60.0	0.00	8	60.0	0.00	8	60.0	0.00	8	60.0	0.00	8
	Mean Hindlimb Splay, mm	36.38	3.998	8	37.33	4.748	8	38.50	3.050	8	38.83	4.717	8
Physiological	Body Temperature, °C	38.24	0.540	8	37.93	0.385	8	37.75	0.414	8	38.38	0.249	8
	Body Weight, g	25.55	1.117	8	25.29	1.023	8	25.60	0.996	8	26.65	0.860	8
Sensorimotor	Thermal Response, sec	22.59	9.758	8	19.34	5.182	8	18.55	9.572	8	21.69	11.033	8

N- number of observations
SD- Standard Deviation

Study title: A Respiratory Safety Evaluation of Orally Administered A-1195425 (ABT-199) in Mice

Study no: TD10-041(R&D/10/317)
Study report location: 4.2.1.3
Conducting laboratory and location: (b) (4) (b) (4).
Date of study initiation: March 11, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: A-1195425 (venetoclax), BREC-0190-085, 15.1 %w/w

Key study findings:
No A-1195425-induced effects on respiratory function were noted, under the conditions tested.

Methods:

Doses:	0, 50, 200, and 600 mg/kg
Frequency of dosing:	Single dose with monitoring up to 6 hours post-dose
Route of administration:	Oral gavage
Dose volume:	20 mL/kg
Formulation/Vehicle:	Vitamin E TPGS and Copovidone [23.5:76.5, %w/w]) in distilled water
Species/Strain:	Crl:CD1®(ICR)
Number/Sex/Group:	8 males
Age:	Approximately 9 weeks
Weight:	30.8 to 34.6 g
Dose justification:	Based on existing preclinical activity, toxicity and pharmacokinetics data

Mortality: All animals survived until termination of the study.

Clinical signs: unremarkable

Respiratory rate, Tidal volume and Minute volume: unremarkable

Figure 11: Mean Respiratory Rate in Male Mice Administered Venetoclax Orally

(Excerpted from Applicants NDA)

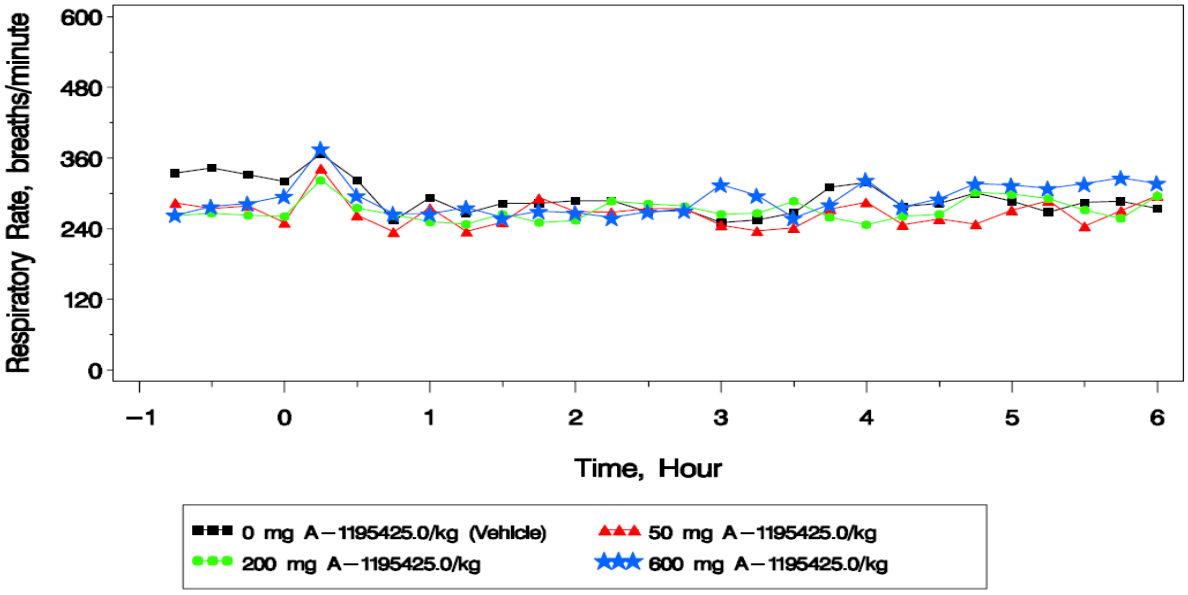


Figure 12: Mean Tidal Volume in Male Mice Administered Venetoclax Orally

(Excerpted from Applicants NDA)

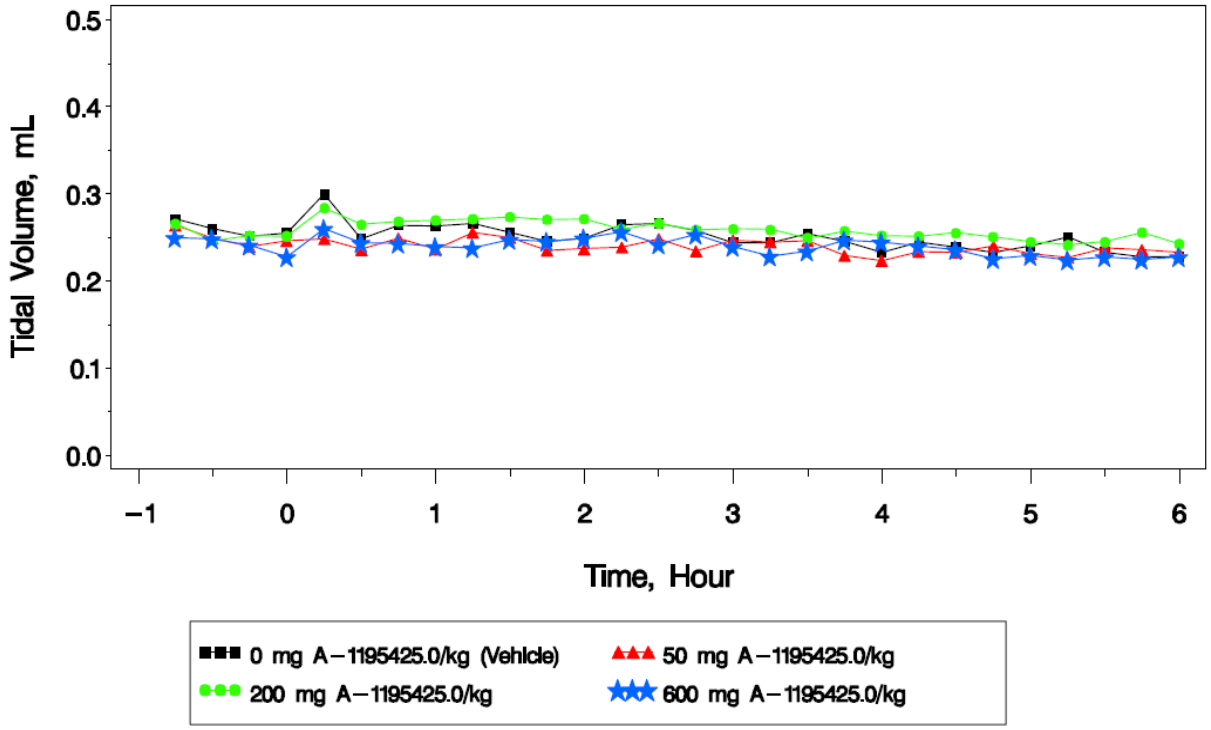
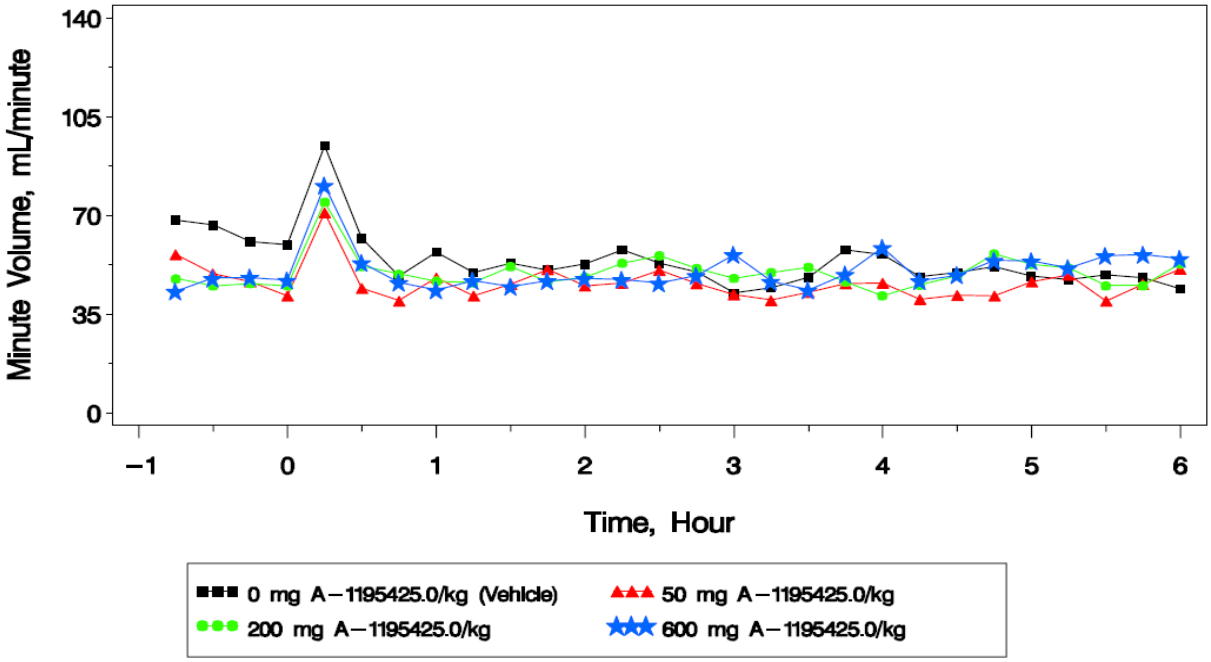


Figure 13: Mean Minute Volume in Male Mice Administered Venetoclax Orally

(Excerpted from Applicants NDA)



Study title: A Cardiovascular Safety Evaluation of Orally Administered A-1195425(ABT-199) in Beagle Dogs

Study no:	TD10-042 (R&D/10/318)
Study report location:	4.2.1.3
Conducting laboratory and location:	(b) (4). (b) (4).
Date of study initiation:	March 11, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1195425.0 (venetoclax) (b) (4) Lot# BREC-0190-085, 15.1 %w/w

Key study findings: No toxicologically significant venetoclax-induced effects were seen on arterial pressure, heart rate, body temperature, or electrocardiographic intervals, under the conditions tested.

Methods:

Doses:	0, 5, 50, and 150 mg/kg
Frequency of dosing:	Single dose with monitoring up to 20 hours post-dose
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	Vitamin E TPGS and Copovidone [23.5:76.5, %w/w]) in distilled water
Species/Strain:	Dog / Beagle
Number/Sex/Group:	6 males
Age:	1-2.5 years
Weight:	10 to 13 kg
Satellite groups:	None
Dose justification:	Based on existing preclinical activity, toxicity and pharmacokinetics data.

Observations and Results:
The animals were surgically instrumented with radio telemetry transmitters for measurement of body temperature, blood pressure, heart rate, and the electrocardiogram (ECG).

Mortality: All animals survived to study termination.

Clinical signs: Vomitus (described as yellow/frothy, tan/food-like, or yellow/food-like) was observed following the 50 and 150 mg A-1195425.0/kg treatments.

Body weight: unremarkable

Body temperature: unremarkable

Heart rate: unremarkable

Mean arterial pressure: unremarkable

Systolic pressure: unremarkable

Diastolic pressure: unremarkable

Electrocardiograph: unremarkable

QT/QTc intervals: unremarkable

Study title: A-1195425: In Vitro Effects on hERG Current

Study no:	R&D/10/795
Study report location:	4.2.1.3
Conducting laboratory and location:	Not provided
Date of study initiation:	June 8, 2009
Drug, lot #, and % purity:	A-1195425.0, Lot#: 1689270, purity

Key study findings:
Under the conditions tested, venetoclax has a low potential to induce QT prolongation.

Methods:

Strains/species/cell line:	Human Embryonic Kidney Cells
Controls:	Vehicle: Dimethyl Sulfoxide (DMSO) Reference: E-4031
Concentrations:	10 μM (Mean bath concentration attained was 1.76 μM (1528 ng/mL)
Test system:	Standard hERG assay

Results:
A-1195425 significantly reduced hERG tail current compared to vehicle-treated cells at the concentration tested. The IC50 value for hERG block was estimated to be ~9987 ng/mL (11.5 μM).

Table 12: Effect of A-119425 on hERG Current

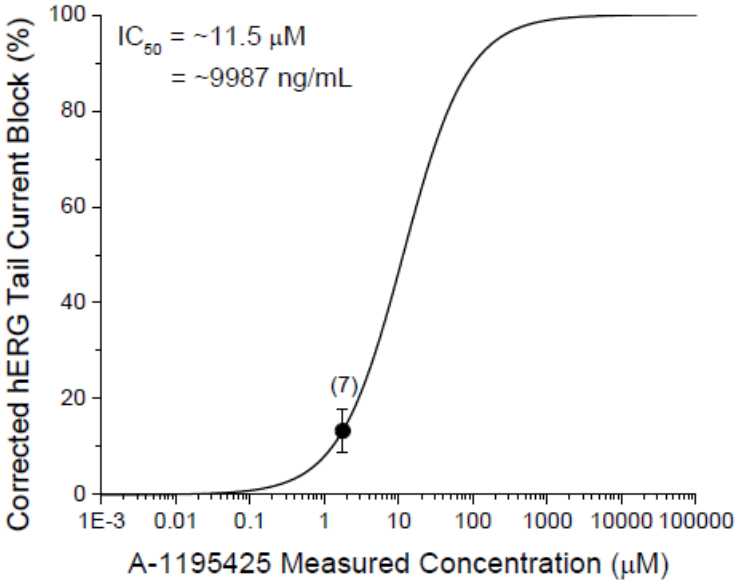
(Excerpted from Applicant's NDA)

	A-1195425						*P* Values
	Measured Drug Concentration (μM)	% Current Blocked	n	Vehicle (DMSO) Concentration (% [vol.])	% Current Blocked	n	Difference (*vs.* DMSO)
Tail Current	1.76	19.17 ± 4.40	7	0.1	6.77 ± 2.50	7	0.0353*
Activating Current	1.76	-24.67 ± 8.03	7	0.1	-22.68 ± 4.01	7	0.8299

*$P < 0.05$, unpaired t-test

Figure 14: Effect of A-1195425 on hERG Tail Current

(Excerpted from Applicant's NDA)



5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Reviewed by Emily Place, PhD

Absorption

Study title: A-1195425 Pharmacokinetics Following Intravenous or Oral Dosing in Mouse

Study no.:	Memo No. 3
Study report location:	4.2.2.2.
Conducting laboratory and location:	Abbvie, Inc. Chicago, IL 60064
Stud Report Date:	April 2009 to July 2014
Drug, lot #, and % purity:	A-1195425.0 (venetoclax), Lot #1689270-0.

Methods

Formulation in solution was a ratio of DMSO: EtOH: Cremophor EL:D5W (2.5:5:10:20:67.5, by volume). Samples were collected using reversed phase high performance liquid chromatography with tandem mass spectrophotometry (LC/MS/MS). The lower limit of quantitation (LLOQ) was 7.00 ng/mL.

Key Findings:

- Absorption of venetoclax was moderate following oral dosing (t_{max} 4 h).
- The mean plasma C_{max} value was 2.57 µg/mL for venetoclax following oral dosing.
- The mean plasma $AUC_{(0-\infty h)}$ value for venetoclax was 19.9 hr*µg/mL following oral dosing.
- The elimination half-life of venetoclax following oral dosing was 2.7 hours.
- Oral bioavailability was 27% (dose-normalized $AUC_{(0-\infty)}$oral/ dose-normalized $AUC_{(0-\infty)}$ IV)

Species/strain.:	CD1 Mice
N:	4 per time point
Dose:	10 mg/kg
Frequency:	Single dose
Volume:	10 mL/kg

Table 13: A-1195425 Pharmacokinetics Following Intravenous or Oral Administration

(Excerpted from Applicant's NDA)

10 mg/kg Intravenous Dose

$t_{1/2}$ (hr)	V_c (L/kg)	V_{ss} (L/kg)	V_β (L/kg)	CL_p (L/hr•kg)	$AUC_{0-\infty}$ (µg•hr/mL)	n
3.5	0.26	0.45	0.69	0.14	74.0	3

10 mg/kg Oral Dose

$t_{1/2}$ (hr)	C_{max} (µg/mL)	T_{max} (hr)	$AUC_{0-\infty}$ (µg•hr/mL)	F (%)	n
2.7	2.57	4.0	19.9	26.8	3

Formulation: solution in 2.5% DMSO: 10% EtOH: 20% Cremophor EL: 67.5 % D5W (by volume)
n=3 mice per time point

Study title: A-1195425 Pharmacokinetics following Intravenous or Oral Dosing in Dog

Study no.:	Memo No. 6
Study report location:	4.2.2.2.
Conducting laboratory and location:	Abbvie, Inc. Chicago, IL 60064
Study Report Date:	April 2009 to July 2014
Drug, lot #, and % purity:	A-1195425.0 (venetoclax) Lot # 31, 96300-44 (FIH formulation).

Key Findings

- Absorption of venetoclax was slow following oral dosing (t_{max} 9.8 hours +/- 11SD).

- The mean plasma C_{max} and $AUC_{(0-\infty h)}$ was 9.3 µg/mL and 195 hr*µg/mL respectively, for venetoclax following oral dosing.
- The elimination half-life of venetoclax following oral dosing was 14.4 hours.
- Oral bioavailability was 28% following 2.5 mg/kg single dose (dose-normalized AUC0-∞ oral/ dose-normalized AUC0-∞ IV).

Methods

Species/strain.:	Beagle dogs
N:	2 for IV and 3 for oral 2.5 mg/kg dose; 12 using 100 mg dose
Dose:	100 mg
Frequency:	Single dose
Volume:	10 mL/kg

The formulation in solution for the 2.5 mg/kg intravenous and oral dose (0.5 mL/kg) was 10% DMSO in PEG-400. Designed for the first in human trial the formulation for the (b) (4) 100 mg dose in solution was a ratio of (b) (4), by weight). Lot # 096300-31 is a tablet prepared from (b) (4) and lot #96300-44 was a formulated tablet. Both forms were tested notably lot -44 was the formulation used for first in human trials. Samples were collected using reversed phase high performance liquid chromatography with tandem mass spectrophotometry (LC/MS/MS). The lower limit of quantitation (LLOQ) was 7.00 ng/mL.

Observations and times: Samples collected by jugular vein at 0, 15, 30 minutes and 1, 1.5, 2, 4, 6, 9, 12, 15, 24, 30, 48, 72 and 96 hours post dose. For studies using 100 mg flat dose samples were collected at 0, 15, 30 minutes and 1, 1.5, 2, 4, 6, 9, 12, 24, and 48 hours post dose.

Table 14: Venetoclax Pharmacokinetics After IV or Oral Dosing in Dog

2.5 mg/kg Intravenous Dose

Dog #	Protocol#	$t_{1/2}$ (hr)	C_0 (µg/ml)	V_c (L/kg)	V_{ss} (L/kg)	V_β (L/kg)	AUC_∞ (µg•hr/ml)	CL_p (L/hr•kg)	Plt_{min} (K/µl)
Dog 1	V09-0667	12.4	20.0	0.13	0.34	0.37	121.9	0.021	226
Dog 2	V09-0667	11.7	19.8	0.13	0.26	0.29	142.7	0.018	357
	Mean	12.0°	19.9	0.13	0.30	0.33	132.3	0.019	292
	SD		0.1	0.00	0.05	0.05	14.7	0.002	93

2.5 mg/kg Oral Dose

Dog #	Protocol #	$t_{1/2}$ (hr)	C_{max} (µg/ml)	T_{max} (hr)	AUC_∞ (µg•hr/ml)	F (%)	Plt_{min} (K/µl)
Dog 4	V09-0667	11.8	2.56	1.5	43.66	33.0	548
Dog 5	V09-0667	14.4	1.85	2.0	40.27	30.4	246
Dog 6	V09-0667	16.0	1.23	1.5	26.23	19.8	349
	Mean	13.8°	1.88	1.7	36.72	27.8	381
	SD		0.67	0.3	9.24	7.0	154
	SEM		0.38	0.2	5.33	4.0	89

° harmonic mean.

Formulation: 10% DMSO in PEG-400; 0.5 ml/kg

Table 15: Summary Pharmacokinetics of Venetoclax in Dogs Following a Single 100 mg Oral Dose (Solution vs. FIH Tablet Formulations)

(Excerpted from Applicant's NDA)

Solution in Lipid Vehicle		$t_{1/2}$	C_{max}		T_{max}	AUC_∞	
Dog #	Protocol #	(hr)	(µg/mL)	C_{max}/D	(hr)	(µg•hr/mL)	AUC/D
Dog 1	V10-2201-1	9.9	4.50	0.486	2.0	90	9.7
Dog 2	V10-2201-1	14.1	13.30	0.944	12.0	396	28.1
Dog 3	V10-2201-1	16.1	6.49	0.662	9.0	235	23.9
Dog 4	V10-2201-1	21.5	9.68	0.687	12.0	402	28.5
Dog 5	V10-2201-1	13.4	7.28	0.823	9.0	228	25.8
Dog 6	V10-2201-1	18.1	7.70	0.516	12.0	287	19.2
	Mean	14.6°	8.16	0.686	9.3	273	22.5
	SD		3.03	0.176	3.9	117	7.2
	SEM		1.24	0.072	1.6	48	2.9

Tablet (lot 96300-31)		$t_{1/2}$	C_{max}		T_{max}	AUC_∞	
Dog #	Protocol #	(hr)	(µg/mL)	C_{max}/D	(hr)	(µg•hr/mL)	AUC/D
Dog 1	V10-2201-2	15.4	3.09	0.334	1.5	35	3.7
Dog 2	V10-2201-2	19.0	6.92	0.491	12.0	262	18.6
Dog 3	V10-2201-2	15.5	7.43	0.758	1.5	145	14.8
Dog 4	V10-2201-2	17.7	15.00	1.065	3.0	257	18.2
Dog 5	V10-2201-2	24.3	8.84	0.999	2.0	166	18.8
Dog 6	V10-2201-2	19.7	6.94	0.465	24.0	231	15.5
	Mean	18.2°	8.04	0.685	7.3	183	14.9
	SD		3.91	0.303	9.1	87	5.8
	SEM		1.60	0.124	3.7	36	2.4

Tablet (lot 96300-44)		$t_{1/2}$	C_{max}		T_{max}	AUC_∞	
Dog #	Protocol #	(hr)	(µg/mL)	C_{max}/D	(hr)	(µg•hr/mL)	AUC/D
Dog 1	V10-2201-3	9.8	4.80	0.518	3.0	71	7.7
Dog 2	V10-2201-3	11.9	8.18	0.581	24.0	248	17.6
Dog 3	V10-2201-3	18.7	8.23	0.839	2.0	144	14.7
Dog 4	V10-2201-3	18.3	14.0	0.994	3.0	262	18.6
Dog 5	V10-2201-3	16.0	12.3	1.39	3.0	197	22.2
Dog 6	V10-2201-3	16.6	8.52	0.571	24.0	248	16.6
	Mean	14.4°	9.34	0.816	9.8	195	16.2
	SD		3.30	0.336	11.0	75	4.9
	SEM		1.35	0.137	4.5	31	2.0

° harmonic mean; C_{max}/D [µg/mL per mg/kg]; AUC/D [µg•hr/mL per mg/kg]

Period 1: 10 mg/ml solution in PEG-400: cremophor EL: oleic acid (10:10:80, by weight; 10 mL/dog)

Period 2: 100 mg tablet: lot 96300-31: (b) (4) (b) (4)

Period 3: 100 mg tablet; lot 96300-44: (b) (4) (b) (4)

Distribution

Study title: Quantitative Whole-Body Autoradiography of Pigmented Rats Following Oral Administration of ^{14}C-ABT-199

Study no.: R&D/13/052

Study report location: 4.2.2.3.

Conducting laboratory and location: (b) (4) (b) (4).

Study Report Date: February 7, 2013

Drug, lot #, and % purity: ABT-199 (venetoclax), Lot# 89336PP00, Purity 98.8%; ^{14}C-venetoclax, Lot# 10016964-508, Purity 99% (chemical purity), 100% (Radiopurity) 100 µCi/kg

Key findings:

- Exposure based on C_{max} and/ or AUC was highest in the liver, lymph nodes, small intestine, adrenal glands, kidney cortex, kidney, and the pancreas.
- Relatively low amount of radioactivity in the brain with the exception of the pituitary gland and the choroid plexus indicates that drug-related materials did not cross the blood-brain barrier.
- Drug related materials did not bind selectively to melanin containing tissues.

Methods

Doses:	5 mg/kg
Frequency of dosing:	Once
Route of administration:	Oral gavage
Dose volume:	1 mL/kg
Formulation/Vehicle:	2.5% dimethyl sulfoxide (DMSO), 20% Cremophor EL, and 10% ethanol, and 67.5% D5W
Species/Strain:	Long Evans rats (HsdBlu:LE)
Number/Sex/Group:	10 per sex/group 1; 1 per sex/group 2.
Age:	Approximately 7 to 9 weeks
Weight:	176 to 217 g

Tissue distribution of total radioactivity was evaluated by quantitative whole-body autoradiography (QWBA) in male and female Long Evans rats after oral administration of ^{14}C-Venetoclax. Pharmacokinetic parameters and dosimetry projections were calculated for blood, plasma, and tissues. For QWBA blood was collected from one animal/sex/time point up to 192 hours postdose from group 1 and up to 24 hours from group 2.

	Number of Animals		Dose	Target Dose Level	Target Dose Volume	Samples
Group	Male	Female	Route	(mg/kg)	(mL/kg)	Collected
1	10	10	Oral	5	1	Blood and Carcasses for QWBA
2[a]	1	1	Oral	5	1	Carcasses for QWBA

QWBA Quantitative whole-body autoradiography.
Note: The dose was approximately 100 µCi/kg for Group 1.
a Group 2 was a control group and only received the nonradiolabeled test article.

Estimated pharmacokinetic parameters following oral administration of ^{14}C-venetoclax in male and female Long Evans rats are presented in the tables below.

Table 16: Estimated Pharmacokinetic Parameters Used to Calculate Radiation Absorbed Doses for Selected Tissues in Male Long Evans Rats Following a Single Oral Dose of ^{14}C- Venetoclax (Groups 1, 5 mg/kg)

(Excerpted from Applicant's NDA)

Matrix	AUC_{0-t} (ng-eq hr/g)	$AUC_{0-\infty}$ (ng-eq·hr/g)	C_{max} (ng-eq/g)	T_{max} (hr)	$t_{1/2}$ (hr)	r^2-adjusted	Dose Exposure (µCi·hr)	Radiation Absorbed Dose (mRad)[a]
Adrenal gland(s)	15384	20942	3060	2.00	3.74	0.977	0.0732	0.549
Blood	4362	4671	1120	2.00	1.83	0.998	6.38	0.120
Blood (LSC)	4305	4328	836	2.00	3.16	0.944	5.91	0.111
Bone	NC	NC	NC	NC	NC	NC	NC	NC
Bone marrow	4852	6075	918	2.00	3.19	0.869	0.415	0.0335
Brain cerebellum	NC	NC	NC	NC	NC	NC	NC	NC
Brain cerebrum	NC	NC	NC	NC	NC	NC	NC	NC
Brain choroid plexus	2976	4758	556	4.00	4.26	1.00	NC[b]	NC[b]
Brain medulla	NC	NC	NC	NC	NC	NC	NC	NC[b]
Brain olfactory lobe	NC	NC	NC	NC	NC	NC	NC	NC[b]
Bulbo urethral gland	17847	18240	1570	4.00	4.22	0.985	NC[b]	NC[b]
Cecum	8884	9863	569	8.00	6.53	1.00	0.701	NC[b]
Diaphragm	6173	8631	1260	4.00	3.21	1.00	NC[b]	NC[b]
Epididymis	5059	10929	164	8.00	46.8	0.685	0.333	8.73
Esophagus	3790	5096	656	4.00	3.24	1.00	NC[b]	NC[b]
Exorbital lacrimal gland	9151	10168	620	8.00	6.41	1.00	NC[b]	NC[b]
Eye lens	NC	NC	NC	NC	NC	NC	NC	NC
Eye uveal tract	1845	2947	324	4.00	4.42	1.00	0.0538	0.377
Eye(s)	NC	NC	NC	NC	NC	NC	NC	NC
Fat (abdominal)	2129	2920	452	4.00	3.03	1.00	4.11	0.0296
Fat (brown)	4649	5839	867	4.00	2.68	1.00	0.148	0.00107
Harderian gland	8692	10411	372	8.00	17.0	0.922	0.185	NC[b]
Intra-orbital lacrimal gland	8396	9470	566	8.00	6.77	1.00	NC[b]	NC[b]
Kidney cortex	27584	30238	2450	4.00	26.5	1.00	5.00	1.69
Kidney medulla	19418	19856	2260	4.00	4.15	0.988	3.29	1.11
Kidney(s)	23148	24065	2410	4.00	4.85	0.994	3.98	1.35
Large intestine	11695	16511	797	4.00	12.6	0.999	2.23	0.779
Liver	169672	172537	26700	2.00	32.2	0.935	170	9.89
Lungs	6158	7162	1380	2.00	2.54	0.991	0.671	0.0587
Lymph node(s)	26219	28241	5140	4.00	1.58	1.00	NC[b]	NC[b]
Muscle	3003	3941	629	4.00	2.79	1.00	35.0	0.126
Myocardium	7232	8621	1480	2.00	2.75	0.890	0.543	0.173
Nasal turbinates	845	2849	137	4.00	12.6	1.00	NC[b]	NC[b]
Pancreas	19869	20280	1650	4.00	4.17	0.972	1.39	1.04
Pituitary gland	6735	8729	1320	4.00	2.81	1.00	0.00487	0.853
Plasma (LSC)	8002	8056	1540	2.00	8.06	0.643	4.90	0.161
Preputial gland	3534	4223	243	4.00	8.85	0.975	0.0390	NC[b]
Prostate gland	5572	6472	431	4.00	8.02	0.998	0.102	0.629
Salivary gland(s)	17243	17996	1480	4.00	5.07	0.989	0.734	0.907
Seminal vesicle(s)	736	2066	144	4.00	8.78	1.00	0.0637	NC[b]
Skin (nonpigmented)	1321	7659	218	4.00	22.8	1.00	35.3	1.12
Skin (pigmented)	1299	4308	235	4.00	11.3	1.00	19.8	0.631
Small intestine	48405	48928	3750	4.00	3.59	0.934	21.0	3.39
Spinal cord	NC	NC	NC	NC	NC	NC	NC	NC
Spleen	5254	6644	1010	2.00	3.26	0.920	0.341	0.239
Stomach	3109	3933	605	4.00	2.67	1.00	0.394	0.276
Stomach mucosa	1630	NC	779	4.00	NC	NC	0.163	0.114
Stomach wall	468	NC	247	4.00	NC	NC	0.0469	0.0328
Testis(es)	2654	4003	179	4.00	14.0	1.00	0.805	2.41
Thymus	6726	7263	565	4.00	6.10	0.993	0.276	1.16
Thyroid	13376	13707	1500	2.00	4.34	0.997	0.0151	0.0792
Urinary bladder	4010	16482	673	4.00	15.4	1.00	0.0997	0.209

a Calculated for a 100-µCi dose of ^{14}C-ABT-199 in humans.
b No theoretical organ weight and/or S-factor available to calculate this value.
NC = Not calculated

a Calculated for a 100-µCi dose of ^{14}C-ABT-199 in humans.

Table 17: Estimated Pharmacokinetic Parameters Used to Calculate Radiation Absorbed Doses for Selected Tissues in Female Long Evans Rats Following a Single Oral Dose of ^{14}C- Venetoclax (Groups 1, 5 mg/kg)

(Excerpted from Applicant's NDA)

Matrix	AUC_{0-t} (ng-eq·hr/g)	$AUC_{0-\infty}$ (ng-eq·hr/g)	C_{max} (ng-eq/g)	T_{max} (hr)	$t_{1/2}$ (hr)	r^2-adjusted	Dose Exposure (μCi·hr)	Radiation Absorbed Dose (mRad)[a]
Adrenal gland(s)	43319	43942	5450	4.00	8.21	0.737	0.200	1.61
Blood	7393	8021	1280	1.00	1.71	1.00	11.0	0.280
Blood (LSC)	9181	9285	1280	2.00	8.54	0.859	12.7	0.325
Bone	135	1242	55.8	2.00	15.1	1.00	1.21	0.0319
Bone marrow	17345	24214	1520	2.00	56.5	1.00	1.65	0.134
Brain cerebellum	0.00	NC	0.00	NC	NC	NC	NC	NC
Brain cerebrum	0.00	NC	0.00	NC	NC	NC	NC	NC
Brain choroid plexus	13057	14699	1390	4.00	6.86	0.957	NC[b]	NC[b]
Brain medulla	0.00	NC	0.00	NC	NC	NC	NC	NC[b]
Brain olfactory lobe	0.00	NC	0.00	NC	NC	NC	NC	NC[b]
Cecum	19287	20268	999	8.00	10.4	0.923	1.48	NC[b]
Diaphragm	16773	17252	1810	4.00	4.41	0.998	NC[b]	NC[b]
Esophagus	6661	11589	1230	4.00	4.89	1.00	NC[b]	NC[b]
Exorbital lacrimal gland	36310	38617	1920	4.00	11.3	0.998	NC[b]	NC[b]
Eye lens	0.00	NC	0.00	NC	NC	NC	NC	NC
Eye uveal tract	9716	14534	581	4.00	48.4	1.00	0.372	2.60
Eye(s)	316	1301	68.6	4.00	12.4	1.00	0.0333	0.233
Fat (abdominal)	7955	8883	533	8.00	6.55	1.00	12.5	0.0687
Fat (brown)	19727	20528	1720	2.00	5.24	0.944	0.521	0.00286
Harderian gland	30918	35514	962	8.00	33.7	0.890	0.790	NC[b]
Intra-orbital lacrimal gland	32990	36281	1730	4.00	13.0	0.941	NC[b]	NC[b]
Kidney cortex	56529	58757	4640	4.00	27.1	0.996	10.8	4.14
Kidney medulla	31878	40116	3270	4.00	46.4	1.00	7.40	2.82
Kidney(s)	41804	48048	3900	4.00	27.4	1.00	8.86	3.38
Large intestine	25714	27226	1350	4.00	11.5	0.975	3.81	1.38
Liver	226583	228673	21100	4.00	19.5	1.00	211	15.8
Lungs	17987	18706	1920	4.00	4.93	0.962	1.83	0.202
Lymph node(s)	30015	31237	12800	0.500	5.13	0.999	NC[b]	NC[b]
Muscle	5729	9221	1140	4.00	4.15	1.00	81.9	0.491
Myocardium	23433	24036	2560	4.00	4.33	0.991	1.62	0.682
Nasal turbinates	18844	35118	229	48.0	76.2	1.00	NC[b]	NC[b]
Ovary(ies)	12248	13232	936	4.00	6.26	0.986	0.130	1.24
Pancreas	47161	48357	3650	4.00	8.68	0.899	4.03	3.53
Pituitary gland	30117	33643	2850	4.00	27.9	1.00	0.0221	3.87
Plasma (LSC)	15958	16130	2270	2.00	8.49	0.834	9.82	0.322
Preputial gland	14205	15094	736	8.00	11.0	0.961	0.118	NC[b]
Salivary gland(s)	44552	46266	3050	4.00	9.66	0.930	1.83	2.26
Skin (nonpigmented)	5250	5875	374	4.00	7.26	0.974	27.1	1.24
Skin (pigmented)	5282	5755	436	4.00	6.37	0.995	26.5	1.21
Small intestine	40268	40900	3910	4.00	7.81	0.905	20.7	3.62
Spinal cord	0.00	NC	0.00	NC	NC	NC	NC	NC
Spleen	19919	20800	1720	2.00	10.8	0.815	1.08	0.869
Stomach	10162	10769	978	4.00	5.50	0.998	1.13	0.850
Stomach mucosa	3326	NC	1330	4.00	NC	NC	0.350	0.262
Stomach wall	671	NC	391	4.00	NC	NC	0.0706	0.0529
Thymus	17828	19017	937	4.00	11.3	0.950	0.846	4.44
Thyroid	30899	32140	2700	4.00	10.4	0.868	0.0375	0.232

a Calculated for a 100-μCi dose of ^{14}C-ABT-199 in humans.
b No theoretical organ weight and/or S-factor available to calculate this value.
NC = Not calculated

Urinary bladder	8060	8570	613	4.00	5.76	0.966	0.0547	0.144
Uterus	7327	9851	485	4.00	11.6	0.990	0.384	0.505

a Calculated for a 100-μCi dose of ^{14}C-ABT-199 in humans.

Metabolism

Drug Metabolism Memo No. 16
Preliminary Metabolite Identification of A-1195425 (ABT-199) in Plasma Samples from Mouse, Dog and Human

Study no.:	Memo 16
Study report location:	eCTD 4.2.2.2
Conducting laboratory:	AbbVie Inc, North Chicago, IL
Date of study initiation:	Release date 10 August 2009
Drug:	A-1195425 (venetoclax)

Key study findings:

- The metabolites in human plasma account for 21-25% of total drug related materials.
  - Metabolite M27 accounts for 14-19.5% of total drug related material.
  - Eight additional metabolites represent less than 3% of the total drug related material following multiple dosing of venetoclax
- The metabolites in both mouse and dog plasma account for ≤ 13% of total drug related materials.

Species/strain.:	CD1 mice; Beagle dogs
N:	3 mice/sex; 4 dogs/sex
Dose:	300 mg/kg mouse; 20 mg/kg dog
Frequency:	Once daily for 5 days mouse; once daily for 39 weeks dog
Route:	oral gavage both animals
Volume:	15 mL/kg mouse; oral (b) (4) dog

Methods: Metabolites were structurally characterized by HPLC coupled to tandem mass spectrometry (LC/MS/MS). Human plasma samples were obtained from clinical study M12-175 (phase 1); two subjects were given a 200 mg single oral dose of venetoclax. Human plasma samples were collected at 0, 2, 3, 4, 6, 8 and 24 hours post dose and retained individually. Mouse plasma was obtained from study V13-4853, plasma collected on Day 5 was pooled and analyzed. Dog plasma was obtained from study TB12-032, plasma collected on Day 272 was pooled and analyzed.

Table 18: Estimated Amount of Venetoclax and Metabolism in Mouse and Dog Plasma

(Excerpted from Applicant's NDA)

	% of Total Drug-Related Material*			
	Mouse (300 mg/kg)		Dog (20 mg/kg)	
Metabolite	Male	Female	Male	Female
A-1195425	87.0	89.2	94.7	90.9
M1	0.2	0.3	-	0.2
M2	1.1	1.1	3.5	3.2
M3	1.4	0.6	0.7	0.9
M4	0.9	1.0	0.3	0.5
M5	1.7	1.3	0.6	0.7
M6	-	-	-	0.2
M9	-	-	-	0.1
M10	-	-	0.3	2.6
M11	0.02	0.01	-	0.2
M14	0.8	0.3	-	0.5
M17	0.1	0.5	-	-
M18	6.8	5.7	-	-

* calculated using MS peak area, assuming equi-molar response in LC-MS of each component.
- indicates not detected

Table 19: Estimated Amount of Venetoclax and Metabolites in Human Plasma

(Excerpted from Applicant's NDA)

	% of Total Drug-Related Material*			
Metabolite	Single Dose (200 mg)	Multiple Dose (400 mg)	Multiple Dose (600 mg)	Multiple Dose (800 mg)
A-1195425	75.8	78.9	77.3	74.8
M1	3.0	-	-	-
M2	3.5	1.1	0.8	1.0
M3	2.2	0.8	1.0	0.5
M4	3.7	1.2	1.9	1.2
M5	4.4	1.7	2.4	1.6
M9	0.7	-	-	-
M11	1.8	0.2	0.3	-
M14	-	0.1	0.5	0.3
M17	2.9	0.9	0.2	0.5
M18	2.0	1.0	1.7	0.7
M27	-	14.1	14.0	19.5

* calculated using MS peak area, assuming equi-molar response in LC-MS of each component.
- indicates not detected

Drug Metabolism Memo No. 23
Plasma Concentrations of M27 Following Oral Administration of A-1195425 in Mouse and Dog

Study no.:	Memo 23
Study report location:	eCTD 4.2.2.2
Conducting laboratory:	AbbVie Inc, North Chicago, IL
Date of study initiation:	Release date 10 August 2009
Drug:	A-1195425 (venetoclax)

Key Findings

- Metabolite M27 is present at very low levels in mouse and dog plasma.
  - M27 exposures averaged 0.208 and 0.794 as a percentage of the total plasma AUC (M27 + venetoclax) in dog and mouse, respectively.

Species/strain.:	CD1 mice; Beagle dogs
N:	3 mice/sex; 4 dogs/sex
Dose:	600 mg/kg mouse; 100 mg/kg dog
Frequency:	Once daily for 5 days mouse and dog
Route:	oral gavage for mouse' oral tablet for dog
Volume:	20 mL/kg mouse

Blood samples were collected on Day 5 for pharmacokinetic profiling.

Table 20: Summery of Pharmacokinetic Profiling of the Metabolite M27 in Mice and Dogs

(Excerpted from Applicant's NDA)

Species	Study	Dose (mg/kg/day)	C_{max} (µg/mL)		AUC (µg•hr/mL)		M27 % of Total (M27 + ABT-199)	
			ABT-199	M27	ABT-199	M27	C_{max}	AUC
Dog	V14-2687	100	41.0	0.0846	605	1.27	0.228	0.208
Mouse	V14-2688	600	7.83	0.0620	96.7	0.760	0.858	0.794

Table 21: Venetoclax and M27 Plasma Pharmacokinetics Following Five Days of 100 mg/kg/day Oral Dosing with Venetoclax in Dog

(Excerpted from Applicant's NDA)

ABT-199 Pharmacokinetics

ID	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-t} (µg•hr/mL)
D31	59.9	6	814
D32	35.2	6	641
D33	27.9	3	359
Mean	41.0	5.0	605
SD	16.8	1.7	230
n	3	3	3

M27 Pharmacokinetics

ID	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-t} (µg•hr/mL)	%Total*	
				C_{max}	AUC_{0-t}
D31	0.0724	6	1.16	0.121	0.142
D32	0.116	3	2.06	0.328	0.320
D33	0.0655	0	0.584	0.234	0.162
Mean	0.0846	3.0	1.27	0.228	0.208
SD	0.0274	3.0	0.744	0.104	0.0975
n	3	3	3	3	3

* % Total calculated from the sum of ABT-199 + M27 concentrations

Study V14-2687

Table 22: Venetoclax and M27 Plasma Pharmacokinetics following Five Days of 600 mg/kg/day Oral Dosing with Venetoclax in CD-1 Mice

(Excerpted from Applicant's NDA)

ABT-199 Pharmacokinetics

ID	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-t} (µg•hr/mL)
M1	6.45	12	85.0
M2	6.58	3	81.2
M3	9.11	3	124
Mean	7.38	6.0	96.7
SD	1.50	5.2	23.7
n	3	3	3

M27 Pharmacokinetics

ID	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-t} (µg•hr/mL)	%Total*	
				C_{max}	AUC_{0-t}
M1	0.0747	12	0.901	1.14	1.05
M2	0.0513	3	0.543	0.774	0.664
M3	0.0600	6	0.835	0.654	0.669
Mean	0.0620	7.0	0.760	0.858	0.794
SD	0.0118	4.6	0.191	0.256	0.221
n	3	3	3	3	3

* % Total calculated from the sum of ABT-199 + M27 concentrations

Study V14-2688

Excretion

Study title: Absorption, Distribution, Metabolism and Excretion of [^{3}H]A-1195425 in Male CD-1 Mice

Study no.:	R&D/14/0704
Study report location:	4.2.2.2.
Conducting laboratory and location:	Abbvie, Inc. Chicago, IL 60064
Study Report Date:	March 2, 2015
Drug, lot #, and % purity:	A-1195425.0 (venetoclax), Lot# 1714884; [^{3}H] labeled A-1195425 (venetoclax), Lot# 10002531-143)

- Excretion was mainly fecal, with ≥ 90% of the radioactivity recovered in the feces; approximately 0.6% was recovered in the urine.
- Approximately half of the parent drug (50%) was recovered in the feces after oral dosing.

Methods

Doses:	10 mg/kg
Frequency of dosing:	Once
Route of administration:	Oral gavage or slow bolus intravenous (IV) injection
Dose volume:	10 mL/kg
Formulation/Vehicle:	DMSO: ethanol: Cremophor EL: D5W (5% dextrose in water), (2.5:10:20:67.5, by volume)
Species/Strain:	CD-1 mice

Total radioactivity in plasma, urine and cage wash was determined by liquid scintillation counting (LSC). Total radioactivity in blood, feces and tissue was determined by LSC followed by sample combustion or solubilization. [^{3}H]venetoclax and non-radiolabeled venetoclax were dissolved in the vehicle at a final concentration of 1 mg/mL. [^{3}H] venetoclax was given a single 10 mg/kg oral dose to 9 male CD-1 mice followed by 0-24, 24-48 and 48-72 hr intervals of urine and feces collection.

Table 23: Excretion of Radioactivity (0-72h) Following Single Oral Dose in Urine and Feces in Mice (% dose)

(Excerpted from Applicant's NDA)

Sample	Time point	Recovery (% Dose)	
		Mean	SD
Urine	0-24 hr	0.3	0.1
	24-48 hr	0.2	0.1
	48-72 hr	0.1	0.0
	Subtotal	**0.6**	**0.2**
Feces	0-24 hr	85.5	4.0
	24-48 hr	7.9	7.4
	48-72 hr	0.2	0.1
	Subtotal	**93.6**	**3.3**
Cage Wash	0-24 hr	1.8	0.9
	24-48 hr	0.0	0.0
	48-72 hr	0.0	0.0
	Subtotal	**1.8**	**0.9**
Total		**96.0**	**4.0**

Table 24: Recovery of Parent Drug and Metabolites in Fecal Excreta of Mice Following Single Oral Dose

(Excerpted from Applicant's NDA)

	Recovery (% of Dose)							
Timepoint (hr)	A-1195425	M2	M3	M4	M5	M18	Unknown	Total
0-24	44.2	5.3	2.7	6.2	2.7	6.5	17.9	85.5
24-48	4.8	ND	1.1	0.7	ND	ND	1.3	7.9
0-48	49.0	5.3	3.8	6.9	2.7	6.5	19.2	93.4

ND: not detected

Unknown: unknown radioactivity peak which is formed during sample process

Table 25: Distribution of Parent Drug and Metabolites Mouse Plasma Following Single Intravenous dose

(Excerpted from Applicant's NDA)

	Plasma Metabolite Profile (% Total Radioactivity)								
ID	A-1195425	M2	M3	M4	M5	M10	M18	Unknown.	Others
1 hr plasma	55.3	4.0	5.2	5.7	2.7	3.2	16.7	4.3	3.0
2 hr plasma	49.4	3.1	3.7	7.3	5.4	3.3	16.1	6.7	3.5
6 hr plasma	18.4	2.2	1.9	1.6	2.0	7.4	6.9	4.0	55.6
24 hr plasma	8.7	4.0	4.3	5.9	2.8	2.8	30.8	1.5	39.3
AUC pooled plasma	**62.2**	**3.2**	**2.6**	**8.0**	**4.0**	**ND**	**9.5**	**10.6**	**ND**

ND: not detected

Unknown: unknown radioactivity peak which is formed during sample process

Study title: Absorption, Distribution, Metabolism and Excretion of [14C]A-1195425 in Male Beagle Dogs

Study no.: R&D/14/0705
Study report location: 4.2.2.2.
Conducting laboratory and location: Abbvie, Inc.
Chicago, IL 60064
Stud Report Date: November 29, 2015
Drug, lot #, and % purity: A-1195425.0 (venetoclax), Lot# 1714884;

[^{14}C] labeled, A-1195425 (venetoclax), Lot# 10016964-509)

- Excretion was mainly fecal, with 87% of the radioactivity recovered in the feces; approximately 0.1% was recovered in the urine.
- Unchanged parent drug was detected in the feces at 26% after oral dosing.

Methods

Doses:	10 mg/kg
Frequency of dosing:	Once
Route of administration:	Oral gavage
Dose volume:	1 mL/kg
Formulation/Vehicle:	Cremophor RH 40: PEG-400: oleic acid (10:10:80, by weight)
Species/Strain:	Male Beagle Dogs
N:	3 males

Three male beagle dogs each received a single 10 mg/kg oral dose. [^{14}C]venetoclax and non-radiolabeled venetoclax were dissolved in the vehicle at 1 mg/mL concentration. Blood samples, urine and feces were collected 168 hours post dose. Total radioactivity in plasma, urine, and feces were determined by LSC. Plasma and fecal samples were processed for metabolite identification and profiling.

Table 26: Recovery of Venetoclax in Male Beagle Dogs

(Excerpted from Applicant's NDA)

Sample	Time point	% of Dose	
		Mean	SD
Urine	0-24 h	0.1	0
	24-48 h	0.0	0
	48-72h	0.0	N.A.
	72-96h	0.0	N.A.
	96-120h	0.0	N.A.
	120-144h	0.0	N.A.
	144-168h	0.0	N.A.
	Subtotal	**0.1**	**0**
Feces	0-24 h	50.5	3.8
	24-48 h	31.6	10.4
	48-72h	3.3	2.2
	72-96h	0.4	0.3
	96-120h	0.7	0.5
	120-144h	0.4	0.2
	144-168h	0.4	0.6
	Subtotal	**87.4**	**8.4**
Cage Wash	0-24 h	3.0	2.2
	24-48 h	1.0	0.3
	48-72h	0.4	0.2
	72-96h	0.1	0.1
	96-120h	0.1	0.1
	120-144h	0.2	0.1
	144-168h	0.0	0
	Subtotal	**4.8**	**2.4**
	Total	**92.2**	**7.7**

N.A. - not applicable

Table 27: Distribution of Parent Drug and Metabolites Dog Plasma Following Single Oral Dose

(Excerpted from Applicant's NDA)

	% Total Radioactivity						
Sample	A-1195425	M2	M3	M4	M5	M7	M10
6h plasma	85.5	5.4	3.7	MS	2.5	MS	MS
48h plasma	100.0	MS	ND	MS	MS	ND	ND
AUC pooled plasma	100.0	MS	MS	MS	MS	ND	ND

MS: detected by MS; ND: not detected

Summary of Pharmacokinetic Profiles Across Species (Mouse, Dog, Monkey, Rat and Human)

Venetoclax oral systemic bioavailability from a PEG-400 solution formulation was low, ranging from 8.6% in monkey to 12.0% in rat to 26.8% in mouse and 27.8% in dog.

Table 28: Absorption of Venetoclax After Single Dose Across Species

(Excerpted from Applicant's NDA)

Test Article: Venetoclax (A-1195425)

Species	Mouse	Rat	Dog	Monkey	Human
Gender (M/F)	M	M	mixed	F	mixed
Number of Animals	3/timepoint	6, 3	2, 3	3, 3	50 CLL/SLL patients
Feeding Condition	ad libitum	fasted	fasted	fasted	fed
Vehicle/Formulation	(a)/solution	(b)/solution	(b)/solution	(b)/solution	(c)/tablet
Dosing Route	IV, PO	IV, PO	IV, PO	IV, PO	PO
Dose (mg/kg)	10	5	2.5	2.5	50 mg
Sample	plasma	plasma	plasma	plasma	plasma
Analyte	A-1195425	A-1195425	A-1195425	A-1195425	A-1195425
Assay	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS
PK Parameters					
IV: $t_{1/2}$ (hr)°	3.5	4.2	12.0	2.2	
V_{ss} (L/kg)	0.45	0.87	0.30	0.49	
CL_p (L/hr•kg)	0.14	0.22 (0.07)	0.02 (0.00)	0.27 (0.09)	
$AUC_{0-\infty}$ (µg•hr/mL)	74.0	23.9 (5.9)	132.3 (14.7)	9.8 (3.2)	
PO: $t_{1/2}$ (hr)°	2.7	3.3	13.8	2.0	16.6
C_{max} (µg/mL)	2.57	0.26 (0.02)	1.88 (0.67)	0.20 (0.16)	0.26 (0.12)
T_{max} (hr)	4.0	6.7 (4.6)	1.7 (0.3)	4.7 (1.2)	6.2 (3.1)
$AUC_{0-\infty}$ (µg•hr/mL)	19.9	2.9 (0.3)	36.7 (9.2)	0.85 (0.34)	5.3 (3.0)
F (%)	26.8	12.0 (1.3)	27.8 (7.0)	8.6 (3.5)	
Study No.	R&D/10/857	R&D/10/857	R&D/10/857	R&D/10/857	M12-175

Additional Information: data provided as Mean (SD); °half-life provided as the harmonic mean.

(a) Solution in DMSO: EtOH: Cremophor EL: D5W (dextrose 5% in water) (2.5:5:10:20:67.5, by volume); 10 mL/kg

(b) 10% DMSO in PEG-400 (0.5-1 mL/kg)

(c) 50 mg (b) (4) tablet (representative lot 10-005689)

In humans, M27 an oxidative metabolite (M+14 Da), was identified as a major metabolite, representing 12.0% of plasma radioactivity after a single oral dose of [^{14}C]venetoclax. M27 was proposed to be formed via mono-oxidation of venetoclax on the 6-position of the cyclohexenyl moiety to give M5, followed by enzyme-mediated cyclization at the α-carbon of piperazine.

Table 29: Estimated Relative Exposures of Venetoclax and M27 at Steady State in Mouse, Dog and Human Plasma Samples following Oral Doses of Venetoclax

(Excerpted from Applicant's NDA)

Species: Dose	Compound	% Total AUC_{0-24}[b]	C_{max} (µg/mL)	AUC_{0-24} (µg•hr/mL)	Exposure Multiples[c]
Mouse	Venetoclax	99.2	7.38	96.7	2.9x
600 mg/kg/day	M27	0.8	0.062	0.760	0.05x
Dog	Venetoclax	99.8	41.0	605	18.4x
100 mg/kg/day	M27	0.2	0.0846	1.27	0.09x
Human	Venetoclax	70.6	2.18	32.9	
400 mg/day[a]	M27	29.4	0.686	14.1	
Human	Venetoclax	69.4	3.64	63.2	
600 mg/day[a]	M27	30.6	1.38	31.4	

(a) multiple dose sample, at steady state; 5 subjects were selected in the 400 mg group and 5 subjects were selected in the 600 mg group.[5] (b) Relative amounts of M27 were calculated assuming other metabolites minimally contribute to total drug-related materials in plasma. Pending identification and quantitation of additional plasma metabolites, the values derived from the current calculation of % Total AUC represent the maximum percent of the plasma AUC that M27 will represent at steady state. (c) Exposure multiples were estimated by dividing steady state exposures in mouse or dog by human steady state exposures at 400 mg dose. N=3 in each species.[37]

At steady state, M27 represents up to 29.4% and 30.6% of M27 + venetoclax exposure at steady state in CLL or NHL patients who received a final dose of 400 and 600 mg/day of venetoclax treatment, respectively. M27 is present in plasma of preclinical toxicology species in vivo at maximal tolerated doses (MTD) but at lower exposure than in human. M27 is not considered a human unique metabolite but is disproportionate. In addition, M30 (reduction of nitrophenyl) and M34 (oxidation, sulfation plus reduction of nitrophenyl) were identified as main metabolites only in human feces. Other minor metabolites including M2, M3, M4, M5, M6, M10, M11, M14, M17 and M18 were also identified in plasma in human and mostly observed in nonclinical species (mouse, rat, rabbit, and dog).

The following table provides venetoclax metabolites in plasma at steady state are compared across species.

Table 30: Venetoclax Metabolite Profile in Plasma at Steady State

(Excerpted from Applicant's NDA)

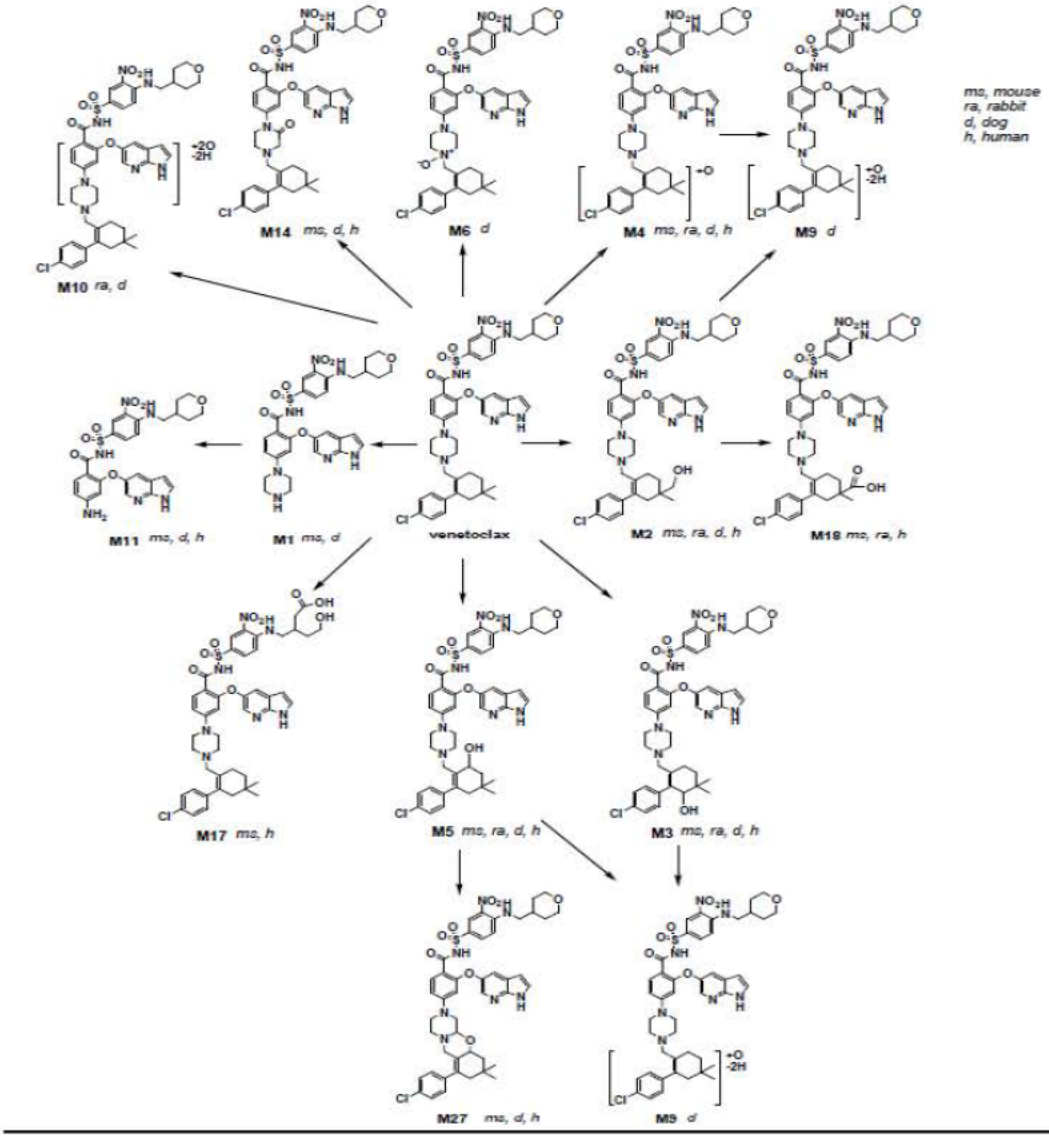
Compound	Mouse	Rabbit	Dog	Human
Venetoclax (A-1195425)	**major**	**major**	**major**	**major**
M1 (loss of chlorophenyl dimethyl cyclohexenyl moiety; A-1610478)	minor		minor	
M2 (dimethyl cyclohexenyl oxidation at methyl group)	minor	minor	minor	minor
M3 (dimethyl cyclohexenyl oxidation; A-1612542)	minor	minor	minor	minor
M4 (dimethyl cyclohexenyl oxidation)	minor	minor	minor	minor
M5 (dimethyl cyclohexenyl oxidation; A-1617595)	minor	minor	minor	minor
M6 (*N*-oxidation of piperazine ring; A-1548065)			minor	
M9 (oxidation, dehydrogenation of cyclohexenyl moiety)			minor	
M10 (dioxidation, dehydrogenation)		minor	minor	
M11 (pyrrolopyridinyl aniline of M1)	minor		minor	minor
M14 (piperazine ring oxidation; A-1572074)	minor		minor	minor
M17 (oxidation on α-methylene of tetrahydropyranyl ring)	minor			minor
M18 (carboxylic acid from M2)	minor	minor		minor
M27 (piperazine α-carbon cyclization from M5; A-1621332))	minor		minor	**major**

Designations of major and minor reflect the FDA MIST and ICH M3 guidelines, where major defined as >10% of total drug related materials in plasma and minor is defined as <10% of total drug related materials in plasma.

A pathway of venetoclax plasma metabolites at steady state is presented in Figure below.

Figure 15: Proposed Biotransfomration Path for Venetoclax in Mouse, Rabbit, Dog and Human Plasma at Steady State

(Excerpted from Applicant's NDA)



6 General Toxicology

6.2 Repeat-Dose Toxicity

Study title: 26-Week Oral Gavage Toxicity Study Of A-1195425 (b) (4) in CD-1 Mice

Study no.:	R&D/12/522
Study report location:	4.2.3.2.
Conducting laboratory and location:	(b) (4). (b) (4)
Date of study initiation:	November 27, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1195425 or venetoclax (A-1195425.0 (b) (4) Lot # 97059-1, 99.1% pure

The bulk test article consisting of (b) (4) venetoclax (as the active pharmaceutical ingredient) (b) (4) Copovidone, (b) (4) Tween 80, and (b) (4) silicon dioxide (w/w). A correction factor of 8.410 was used to adjust for potency when preparing venetoclax formulations.

Key Study Findings

- Test article related early deaths occurred in one animal at 15 mg/kg/day and another at 300 mg/kg/day.
- Target organs of toxicity included lymphoid organs and red cell mass (dose-related decrease RBC mass).

Methods

Doses:	0, 15, 50, and 300 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Placebo (b) (4) consisting of (b) (4) (w/w),
Species/Strain:	Crl:CD1®(ICR) mice
Number/Sex/Group:	30 per sex/group
Age:	Approximately 6 weeks
Weight:	Males: 26.2 to 37.9 g; Females: 22.5 to 29.1 g
Satellite groups:	5 per sex in control and 10 per sex in the test article treated groups for toxicokinetic study

Observations:

Clinical Findings	Weekly
Body weights	Weekly
Food consumption	Weekly
Ophthalmology	Prior to the terminal necropsy
Hematology	Prior to the terminal necropsy
Clinical chemistry	Prior to the terminal necropsy
Gross pathology	Terminal necropsy
Organ weights	Terminal necropsy
Histopathology	Terminal necropsy
Whole blood	Test article concentration analysis; For the control group, samples were collected one hour after dosing on Days 1 and 182. For treated groups samples were collected at 1, 3, 6, 12, and 24 hours postdose on Days 1 and 182. The TK parameters were determined for A-1195425 from mean concentration-time data.

Mortality

Main study mortality			
Dose Group	**Animal Number/Sex**	**Day of Death/Fate**	**Preliminary Cause of Death (Microscopically)**
0 (Control)	1018/M	Day 14/found dead	Probable dosing injury
15	2029/M	**Day 59/found dead**	Undetermined
15	2510/F	Day 11/euthanized in extremis	Accidental injury
15	2521/F	Day 164/found dead	Consistent with dosing injury
50	3017/M	Day 100/euthanized in extremis	Urogenital inflammation
50	3021/M	Day 159/euthanized in extremis	Skin inflammation/necrosis
300	4023/M	**Day 79/found dead**	Undetermined
300	4025/M	Day 62/euthanized in extremis	Undetermined
300	4028/M	Day 107/found dead	Gastrointestinal ulceration possible dosing injury)
300	4507/F	Day 29/euthanized in extremis	Probable dosing injury
300	4511/F	Day 70/euthanized in extremis	Accidental injury

Animal number 4023 had yellow fluid in the abdominal cavity upon gross examination, and microscopic examination revealed lymphoid single cell necrosis in the thymus that was consistent with stress; moderate autolysis precluded definitive microscopic assessment; The death of animal # 4023 and 2029 (No significant clinical observations were present in these animals prior to death) may be related to test article administration.

Clinical Signs
Unremarkable

Body Weights
Unremarkable

Food Consumption
Unremarkable

Ophthalmology
Unremarkable

Hematology

- On Days 92 and 183 in both sexes receiving ≥ 15 mg/kg/day, there were moderate to marked dose-responsive decreases in mean lymphocytes (up to 75%) that resulted in decreases in mean total leukocytes (up to 67%) relative to control means.
- On Days 92 and 183 in both sexes receiving 50 and 300 mg/kg/day, there were minimal to mild dose-related decreases in mean red cell mass (up to 13% relative to controls).

Table 31: Percentage Change in the Hematology Parameters In the Venetoclax Treated Animals Compared to Mean Control Values - 26-week study in Male Mice

RBC Parameters, Males										
Dose, mg/kg	Hemoglobin g/dL		Hematocrit %		MCV fL		MCH pg		RDW %	
	D 92	D 183	D 92	D 183	D 92	D 183	D 92	D 183	D 92	D 183
0	15.16	14.72	49.90	50.11	49.33	50.71	14.98	14.91	12.63	12.88
15	6.7	-1.3	6.8	-2.0	1.2	0.0	1.1	0.8	-2.2	-0.5
50	-4.0	-7.8*	-4.3	-6.7*	2.0	-1.0	2.4	-2.1	2.7	5.5
300	-13.3*	-13.7*	-11.2*	-12.7*	-4.5*	-5.1*	-6.6*	-6.0*	15.8*	11.1*

WBC Parameters, Males						
Dose, mg/kg	Leukocytes $10^3/\mu L$		Lymphocytes $10^3/\mu L$		Eosinophils $10^3/\mu L$	
	D 92	D 183	D 92	D 183	D 92	D 183
0	10.66	9.54	7.629	6.262	0.359	0.268
15	-38.3*	-45.6*	-48.2*	-46.4*	-54.6*	-40.3
50	-60.8*	-53.2*	-69.5*	-61.3*	-60.4*	-54.9*
300	-67.3*	-64.9*	-73.4*	-72.9*	-57.1*	-53.0*

*Significantly different from control

Table 32: Percent Change in the Hematology Parameters in the Venetoclax Treated Animals Compared to Mean Control Values -26-week study in Female Mice

RBC Parameters, Females										
Dose, mg/kg	Hemoglobin g/dL		Hematocrit %		MCV fL		MCH pg		MCHC%	
	D 92	D 183	D 92	D 183	D 92	D 183	D 92	D 183	D 92	D 183
0	15.51	14.76	49.87	49.65	50.33	51.26	15.65	15.23	31.09	15.23
15	-0.1	-2.6	-0.4	-4.6	0.6	-1.1	0.9	1.0	0.1	1.0
50	-2.4	-4.0	-3.1	-4.5*	-1.5	-1.2	-0.8	-0.8	0.6	-0.8
300	-11.2*	-13.1*	-6.6*	-11.2*	-4.8*	-6.5*	-9.5*	-8.6*	-5.0*	-8.6

WBC Parameters, Females						
Dose, mg/kg	Leukocytes $10^3/\mu L$		Lymphocytes $10^3/\mu L$		Basophils $10^3/\mu L$	
	D 92	D 183	D 92	D 183	D 92	D 183
0	8.91	10.26	6.853	7.320	0.016	0.021
15	-31.4*	-42.9*	-39.1*	-48.4*	-56.3	-71.4*
50	-53.3*	-52.1*	-61.9*	-64.2*	-62.5	-71.4*
300	-52.6*	-65.38*	-66.5*	-74.9*	-62.5*	-66.7*

*Significantly different from control

Clinical Chemistry
Unremarkable

Gross Pathology
Unremarkable

Organ Weights

Table 33: Significant Changes in Organ Weight at the End of Dosing - 26-week Mouse study

(Excerpted from Applicant's NDA)

Absolute Organ Weights, Interim: Control Means (g) and Percentage Changes from Control

	Males				Females			
A-1195425 mg/kg/day:	0	15	50	300	0	15	50	300
Spleen	0.115	-29*	-28*	-35*	0.124	-19	-22*	3
Thymus	0.036	17	19	-11	0.048	-4	13	-8

n=10 per group; * indicates statistical significance compared to control data.

Absolute Organ Weights, Terminal: Control Means (g) and Percentage Changes from Control

	Males				Females			
A-1195425 mg/kg/day:	0	15	50	300	0	15	50	300
Spleen	0.105	-29*	-22	-18	0.119	-9	-16	-12
Thymus	0.035	-3	-3	-11	0.047	-23*	-19	-26*

n=17 to 20 per group; * indicates statistical significance compared to control data.

Histopathology
Adequate Battery
Yes.

Peer Review
Yes.

Histological Findings
Test article-related microscopic findings were decreased lymphocytes in lymphoid organs including the mandibular and mesenteric lymph nodes, and spleen, and gut-associated lymphoid tissue (GALT).

Table 34:Histopathology Findings at 26-week Sacrifice in Mice

			Males				Females			
		Dose mg/kg/day	0	15	50	300	0	15	50	300
Tissue		Severity								
GALT	decreased lymphocytes	- minimal	1	0	0	1	0	1	0	4
lymph node, mandibular	decreased lymphocytes	- minimal	0	3	3	2	0	4	5	9
		- mild	3	6	7	6	0	3	5	2
		- moderate	0	0	0	0	0	0	0	4
lymph node, mesenteric	decreased lymphocytes	- minimal	0	0	2	3	1	7	11	5
		- mild	0	2	4	7	0	4	5	9
		- moderate	0	0	0	2	0	0	3	3
Spleen	decreased lymphocytes	- minimal	0	0	3	10	0	1	2	9
		- mild	0	0	0	0	0	0	2	5
	hematopoiesis, extramedullary, increased	- minimal	0	4	10	11	1	3	4	15
		- mild	0	0	0	2	1	0	0	1

Toxicokinetics

- The exposure (AUC_{0-24} and C_{max}) was similar in both males and females on Day 182.
- The exposure (AUC) appeared to be approximately proportional to dose for both sexes at all three dose level on Day 182.
- The average T_{max} occurred approximately between 1 and 3 hours post dosing.

Table 35: Exposure of Venetoclax in Mouse Blood: Day 182

(Excerpted from Applicant's NDA)

Mean Toxicokinetic Parameters for A-1195425				
		Day 182		
A-1195425 Dose Level (mg/kg/day)	Sex	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)
15	Male	0.286	1.0	1.49
	Female	0.269	1.0	1.49
	Overall	0.278	1.0	1.49
50	Male	0.550	3.0	4.42
	Female	0.553	3.0	3.78
	Overall	0.551	3.0	4.10
300	Male	1.67	3.0	17.2
	Female	1.29	3.0	11.8
	Overall	1.48	3.0	14.5

Dosing Solution Analysis
The results for venetoclax concentration ranged from 92 to 100% of the theory for all treatment groups (analyses encompassed the first preparation on Week 1, then monthly intervals, and the last preparation). The results of the homogeneity of dose formulations prepared for Week 1 showed the relative standard deviation of venetoclax concentrations ranged from 0.0 to 0.6% within each treatment group, indicating that the preparations were homogeneous.

Study title: 39-Week (Oral [(b) (4)] Dose) Toxicity Study of A-1195425 [(b) (4)] In Beagle Dogs

Study no.:	R&D/12/384
Study report location:	4.2.3.2.
Conducting laboratory and location:	[(b) (4)]. [(b) (4)]
Date of study initiation:	March 26, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1195425 or venetoclax (A-1195425.0 [(b) (4)] Lot # 97059-1, 99.1% pure

The bulk test article consisting of [(b) (4)] venetoclax (as the active pharmaceutical ingredient), [(b) (4)] Copovidone, [(b) (4)] Tween 80, and [(b) (4)] silicon dioxide (w/w). A correction factor of 8.410 was used to adjust for potency when preparing venetoclax formulations.

Key Study Findings

- All doses of venetoclax were tolerated.
- Hair discoloration starting from Days 95/96 was observed in all males and females at 6 and 20 mg/kg/day.
- Dose-related body weight reductions (up to 15%) correlated with decrease in food consumption.
- Target organs included lymphoid organs, testes, epididymides, skin, liver and pylorus of the stomach.
    - decreased lymphocytes in peripheral blood and in lymphoid tissues
    - moderate to severe, dose dependent bilateral degeneration/atrophy of seminiferous tubules
    - minimal to mild single cell necrosis in the pyloris of the stomach

Methods

Doses:	0, 2, 6, and 20 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral [(b) (4)] dose
Dose volume:	Not applicable
Formulation/Vehicle:	Placebo [(b) (4)], consisting of [(b) (4)]

[redacted] (b) (4)
(w/w) (96457-24-Placebo).
Species/Strain: Beagle dogs.
Number/Sex/Group: 4 per sex/group
Age: Approximately 7 to 8 months of age at receipt
Weight: Males: 7.60 to 8.85 kg; Females: 6.40 to 7.60 kg

Clinical Findings	Twice weekly
Body weights	Twice weekly
Food consumption	Twice weekly
Ophthalmoscopy	During week 17, 21 and prior to necropsy
Hematology	Predose; prior to dosing on Days 2, 28, 90, 181, and 272
Clinical chemistry	Predose; prior to dosing on Days 2, 28, 90, 181, and 272
Gross pathology	Prior to the terminal necropsy
Organ weights	Prior to the terminal necropsy
Histopathology	Terminal necropsy
Plasma Analysis/TK	Predose; 1, 3, 6, 12, and 24 hours after dosing on Days 1, 28, 90, 181, and 272
Peripheral Blood Leukocyte Analysis	Predose; Days 2, 28, 90, 181, and 272

Mortality
All animals survived to the scheduled necropsy.

Clinical Signs
Hair discoloration starting from Days 95/96 was observed in all males and females at 6 and 20 mg/kg/day. The discoloration was observed in the areas of the face first and evolved to include sites such as the cranial region, ears, cervical region, dorsal region, forelimbs/hind limbs, lumbar region, thoracic region, abdominal region, and/or entire body.

Body Weights
Reductions in group mean body weights and body weight gains, ≥5% (versus controls) were noted at 2 (males only), 6 and 20 mg/kg/day. At the end of the dosing period, decreased mean body weight gain was -16.5% (males) and -16.7% (females) at 2 mg/kg/day; and ranged from -28.6% to -53.0% for males and females at ≥ 6 mg/kg/day.

Figure 16: Mean Body Weights During the Dosing Period - 39-week Dog Study-Male

(Excerpted from Applicant's DNA)

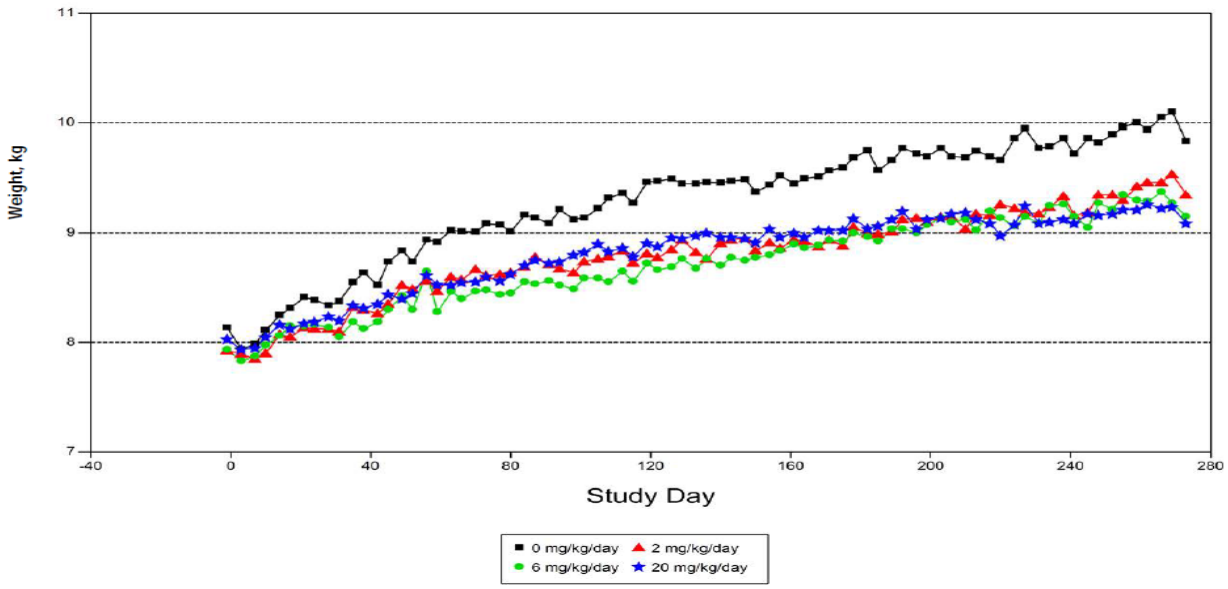
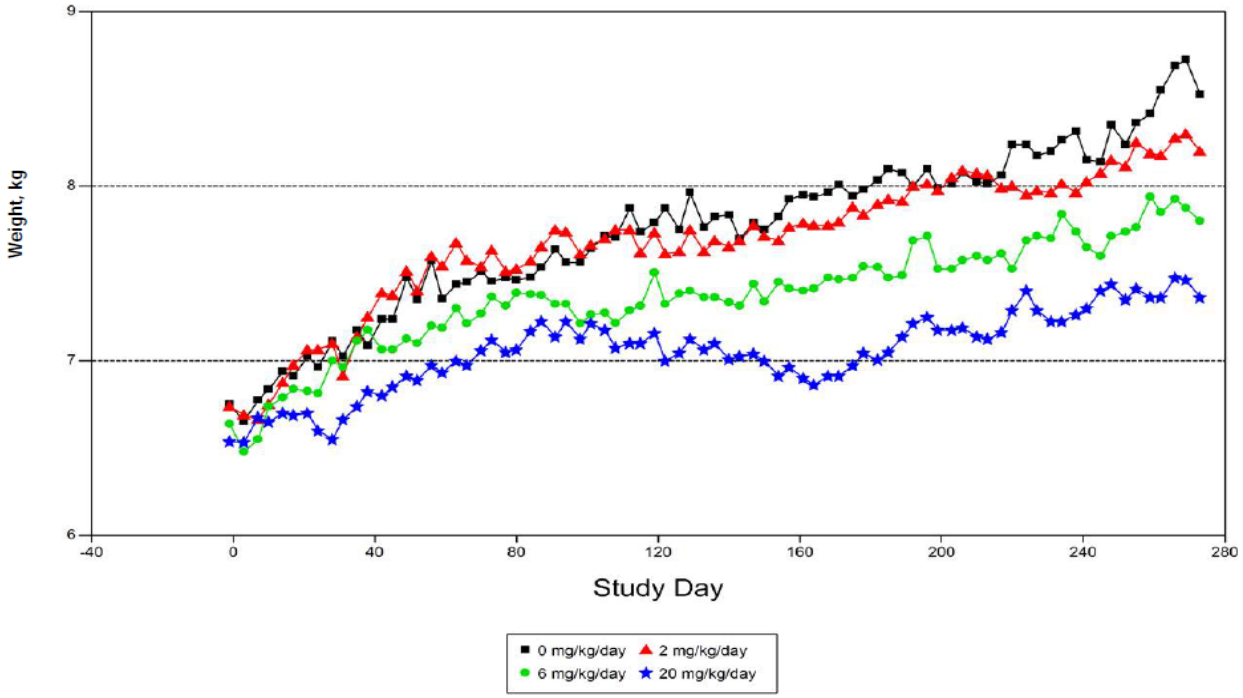


Figure 17: Mean Body Weights During the Dosing Period - 39-week Dog Study-Female

(Excerpted from Applicant's DNA)



Food Consumption

Dose dependent decrease (>10%) in the test article groups was observed compared to control. Decreases of ≥10% were observed as follows: 29% for females at 2 mg/kg/day; 49% for females at 6 mg/kg/day; 29% for males and 51% for females 20 mg/kg/day.

Figure 18: Mean Food Consumption During the Dosing Period - 39-week Dog Study- Male

(Excerpted from Applicant's DNA)

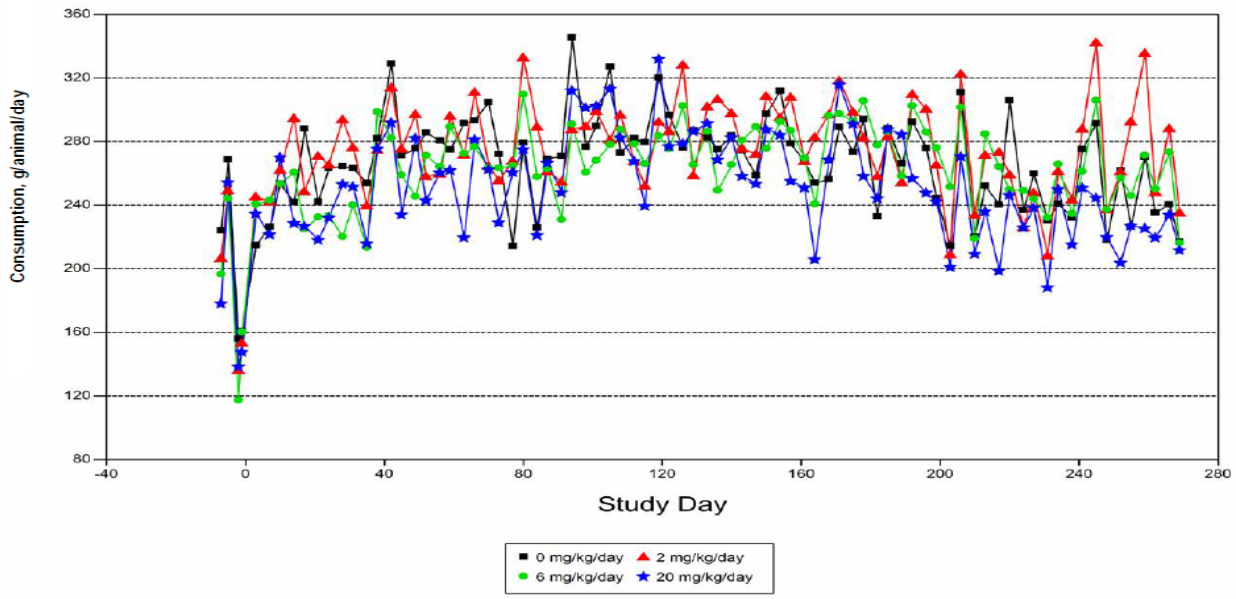
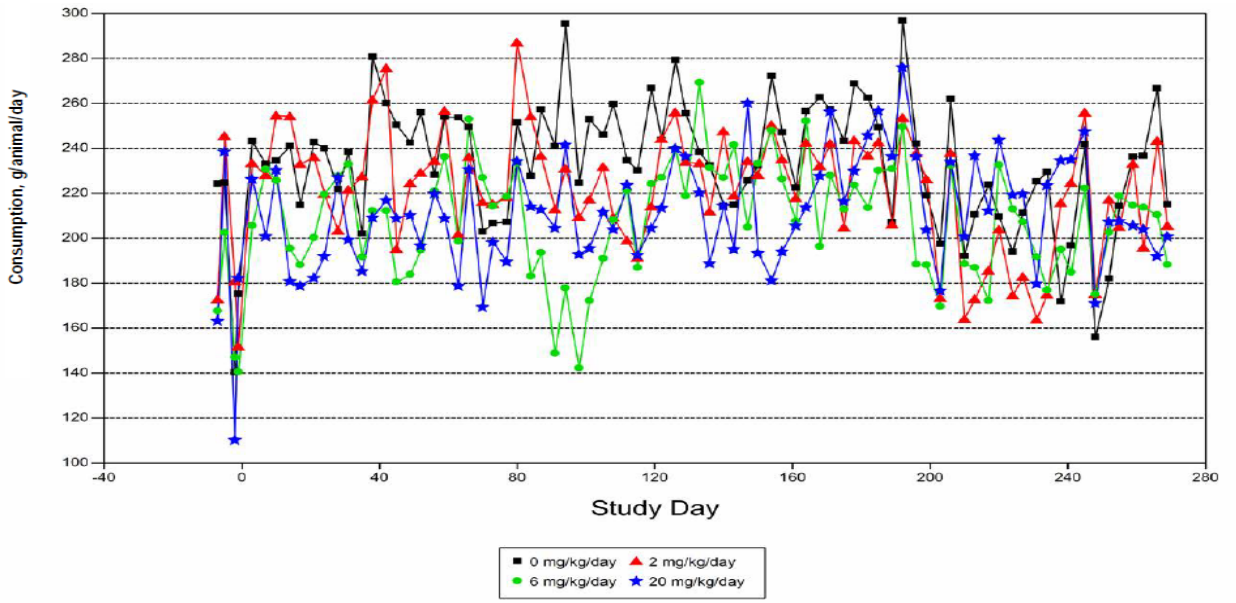


Figure 19: Mean Food Consumption During the Dosing Period - 39-week Dog Study- Female

(Excerpted from Applicant's DNA)



Ophthalmoscopy
Unremarkable

Hematology

Table 36: Mean % Change in the Hematology Parameters Compared to Control Values - 39- Week Dog Study

		Males				Females			
Dose mg/kg/day		0	2	6	20	0	2	6	20
Leukocytes	Day 2	8.4	-9.8	0.0	-13.1	10.4	-16.0	-29.9	-26.0
10^3/µL	Day 28	9.35	-5.9	-10.4	-45.7*	9.4	-8.5	-14.0	-28.7
	Day 90	8.95	2.0	-14.2	-34.3*	9.58	-16.7	-29.2	-8.7
	Day 181	9.28	-19.2	-19.4	-43.1*	9.63	-35.1	-19.5	-19.2
	Day 272	8.03	-3.5	-16.6	-23.7*	9.83	-34.1*	-34.6*	-37.6*
Erythrocytes 106/µL	Day 2	6.475	1.0	-0.7	8.1	6.7	-0.3	-2.5	-3.1
	Day 28	6.983	5.7	6.3	13.2*	7.035	5.3	1.2	6.5
	Day 90	7.143	-1.0	-2.0	4.9	6.913	0.6	-3.2	0.9
	Day 181	7.1	4.7	-1.0	11.8*	6.863	10.1	4.2	12.2
	Day 272	7.595	5.7	3.0	12.2*	7.138	5.9	5.5	16.3
MCV fL	Day 2	70.9	-2.0	-1.9	-5.4*	69.03	-5.1	0.2	-0.9
	Day 28	70.43	-2.2	-3.2	-6.5*	68.15	-4.7	0.0	-2.9
	Day 90	69.73	-2.8	-3.4	-8.5*	67.3	-5.8	-0.5	-5.9
	Day 181	69.93	-3.0	-5.0*	-7.8*	68.07	-4.8	-1.4	-7.4
	Day 272	70.05	-3.5	-5.9*	-10.2*	67.05	-3.2	0.1	-7.7
MCH pg	Day 2	23.38	-2.5	-2.1	-5.0*	22.58	-18.6	-35.7	-33.1
	Day 28	23.5	-3.6	-3.4	-7.7*	22.5	-40.3	-56.4	-66.3
	Day 90	23.43	-2.0	-4.1	-8.5*	22.48	-40.0	-57.8	-67.3
	Day 181	24.2	-3.1	-4.4*	-8.8*	23.48	-52.3	-61.5	-68.7
	Day 272	23.6	-3.4	-5.7*	-8.3*	22.65	-57.3	-64.3	-72.7*
Lymphocytes 10^3/µL	Day 2	2.735	-19.6	-28.7	-25.0	3.143	-18.6	-35.7*	-33.1*
	Day 28	3.078	-30.5*	-55.6*	-69.0*	3.2	-40.3*	-56.4*	-66.3*
	Day 90	3.015	-30.3*	-60.9*	-70.8*	2.918	-40.0*	-57.8*	-67.3*
	Day 181	3.17	-44.6*	-68.3*	-70.6*	3.07	-52.3*	-61.5*	-68.7*
	Day 272	2.523	-40.2*	-65.2*	-69.5*	2.873	-57.3*	-64.3*	-72.7*
Basophils 10^3/µL	Day 2	0.063	3.2	-12.7	42.9	0.093	-26.9	-24.7	-32.3
	Day 28	0.078	-23.1	-44.9	-64.1*	0.058	-22.4	-13.8	-43.1
	Day 90	0.103	-32.0	-56.3*	-72.8*	0.065	-38.5	-26.2	-56.9*
	Day 181	0.085	-2.4	-58.8	-61.28*	0.06	-28.3	-28.3	-61.7*
	Day 272	0.078	-48.7*	-51.3*	-57.7*	0.065	-46.2	-38.5	-61.5

*Significantly different from control

Coagulation
Unremarkable

Clinical Chemistry
Unremarkable

Urinalysis
Unremarkable

Gross Pathology
Test article-related macroscopic findings were seen in the hair (in all males and females at 6 and 20 mg/kg/day, with dose-dependent increasing severity) and small testes of treated males.

Table 37: Summary of Macroscopic Observations at the End of Dosing - 39-week Dog study

Tissue Observation		Severity	0 mg/kg/day	2 mg/kg/day	6 mg/kg/day	20 mg/kg/day
			M/F	M/F	M/F	M/F
Number of Animals Examined			4	4	4	4
hair	discoloration, white	- mild	0/0	0/0	2/2	0/0
		- moderate	0/0	0/0	2/1	1/0
		- severe	0/0	0/0	0/1	3/4
testes	small	- mild	0/-	3/-	3/-	4/-

M=Males
F=Females

Organ Weights
- Test article-related statistically significant organ weight decreases were seen in the testes and prostate gland.

Table 38: Significant Changes in Organ Weight in Grams (g) Relative to Body Weight at the End of Doing - 39-week Dog Study

Dose (mg/kg/day)	0	2	6	20	0	2	6	20
Organ weights	Males				Females			
Testes	11.47	**4.97***	**5.30***	**5.76***	NA	NA	NA	NA
Testes/BWt %	0.12	**0.05***	**0.06***	**0.06***	NA	NA	NA	NA
Prostate gland	11.72	7.43	7.00	**4.95***	NA	NA	NA	NA
Prostate gland/bWt %	0.12	0.08	0.08	**0.05***	NA	NA	NA	NA
Brain	-	-	-	-	67.92	70.82	73.45	70.10
Brain/BWt %	-	-	-	-	0.80	0.87	**0.96***	**0.97***

*Significantly different from control
NA=not applicable
- =unremarkable

Histopathology

Adequate Battery: Yes.

Peer Review: Yes.

Histological Findings

Test article-related microscopic findings were seen in the testes (moderate to severe dose dependent degeneration/atrophy of seminiferous tubules, bilateral) and epididymides (moderate to severe oligospermia) of males, and the skin (decreased pigment), decreased lymphocytes in mandibular and mesenteric lymph nodes, and GALT, gall bladder (minimal pigment, increased macrophage), liver (minimal neutrophil infiltration, pigment, increased kupffer cell), and pylorus of the stomach (single cell necrosis) in males and females. The finding in the male reproductive organs was characterized by a loss of maturing spermatids, spermatocytes, and spermatogonia, accompanied by a degree of vacuolation of the remaining cells lining the seminiferous tubules and dilation of the tubules. Few to no mature spermatids were present in any of the seminiferous tubules.

Table 39: Incidence and Grade of Histopathology Findings at 39-week Sacrifice in Dogs

(Excerpted from Applicant's NDA)

Dose level: mg/kg/day	0		2		6		20	
Sex	M	F	M	F	M	F	M	F
Number Examined	4	4	4	4	4	4	4	4
Testes								
Degeneration/atrophy, seminiferous tubules, bilateral	0		4		4		4	
- moderate	0		2		0		0	
- severe	0		2		4		4	
Epididymides								
Oligospermia/germ cell debris, bilateral	0		4		4		4	
- moderate	0		1		0		1	
- severe	0		3		4		3	
Skin, interscapular region								
Pigment, decreased	0	0	1	0	2	2	4	4
- minimal	0	0	0	0	2	0	0	0
- mild	0	0	1	0	0	0	1	1
- moderate	0	0	0	0	0	1	1	0
- severe	0	0	0	0	0	1	2	3
Stomach, pylorus								
Necrosis, single cell	0	0	3	3	1	3	3	2
- minimal	0	0	2	2	1	2	3	2
- mild	0	0	1	1	0	1	0	0
Gallbladder								
Pigment, increased macrophage								
- minimal	0	0	2	2	4	2	4	4
GALT								
Decreased lymphocytes								
- minimal	0	0	0	0	1	1	1	3
Liver								
Pigment, increased Kupffer cell								
- minimal	0	0	1	0	0	0	1	2
Lymph node, mandibular								
Decreased lymphocytes								
- minimal	0	0	3	2	1	2	3	3
Lymph node, mesenteric								
Decreased lymphocytes	0	0	3	2	4	4	4	4
- minimal	0	0	3	2	3	3	3	3
- mild	0	0	0	0	1	1	1	1

M - Male
F – Female
GALT – Gut-Associated Lymphoid Tissue

Toxicokinetics

- The plasma concentration data suggest that the exposure on Day 1, 28, 90, 181 and 272 as characterized by AUC_{0-24} and C_{max}, were similar for both male and female dogs.
- The mean exposure (AUC) appeared to be approximately proportional to dose for both sexes at all three dose levels on Day 1, 28, 90, 181 and 272 with the exception of high dose on Day 272 between sexes.
- The average T_{max} occurred approximately between 3-7 hours post dosing at all doses with the exception of high dose on Day 1 with 12 hours.

Table 40: Exposure of Venetoclax in Dog Plasma

(Excerpted from Applicant's NDA)

Collection Interval	Sex	Venetoclax (mg/kg/day) 2	6	20
		Mean C_{max} (µg/mL)		
Day 1	Males	0.700	2.12	2.41
	Females	0.577	1.03	2.07
Day 28	Males	0.770	2.55	4.64
	Females	0.872	3.61	9.83
Day 90	Males	0.877	2.70	2.88
	Females	1.07	4.47	6.35
Day 181	Males	1.5	3.79	2.76
	Females	1.40	2.78	8.06
Day 272	Males	1.25	3.83	2.34
	Females	1.29	3.88	9.40
		Mean $AUC_{0\text{-}24hr}$ (µg•hr/mL)		
Day 1	Males	8.44	29.3	33.5
	Females	8.23	13.4	28.2
Day 28	Males	9.03	31.7	75.3
	Females	10.6	43.5	133
Day 90	Males	9.68	36.3	39.2
	Females	12.7	56.4	96.2
Day 181	Males	17.4	53.4	38.4
	Females	19.2	39.5	121
Day 272	Males	15.1	52.1	32.3
	Females	19.4	52.1	139

Table 41: Exposure of Venetoclax Combined for Males and Females

	Dose	Cmax (µg/mL)	Tmax	AUC (µg*hr/mL)
Day 1	2 mg/kg	0.638	3	8.33
	6 mg/kg	1.58	3.8	21.4
	20 mg/kg	2.24	12	30.9
Day 28	2 mg/kg	0.821	4.3	9.84
	6 mg/kg	3.08	6.8	37.6
	20 mg/kg	7.24	5.6	104
Day 90	2 mg/kg	0.974	3.8	11.2
	6 mg/kg	3.58	4.1	46.3
	20 mg/kg	4.61	3.4	67.7
Day 181	2 mg/kg	1.45	5.6	18.3
	6 mg/kg	3.28	5.3	46.5
	20 mg/kg	5.41	3.4	79.8
Day 272	2 mg/kg	1.27	5.8	17.2
	6 mg/kg	3.85	4.9	52.1
	20 mg/kg	5.87	4.5	85.6[1]

[1]The estimated mean AUCs for females was 139 µg*hr/mL and for males was 32.3 µg*hr/mL.

Peripheral Blood Leukocyte Analysis

- Lymphocytes, Mature B cells, Helper T cells (CD4+), Cytotoxic T cells (CD8+), and Mature T cells demonstrated a biologically significant decrease in absolute cell counts after test articles administration.

Table 42: Summary of Peripheral Blood Leukocyte Analysis Values in 39-week Study in Dog

	Females				Males			
Dose(mg/kg/day)	**0**	**2**	**6**	**20**	**0**	**2**	**6**	**20**
Lymphocytes (cells/µL)								
Pretest 1	1920	1930	1703	1588	1695	1548	1700	1755
Pretest 2	1718	1987	1574	1895	1715	1587	1698	1849
Day 2	1897	1624	1242	1264	1997	1287	1181	1049
Day 28	1637	945	695	512	1628	1006	585	573
Day 90	1615	945	669	530	1805	1208	616	555
Day 181	1885	995	740	585	2242	1151	651	575
Terminal	1227	582	484	396	1604	717	415	411
Lymphocytes (% of gated)								
Pretest 1	22	28	22	23	23	18	22	24
Pretest 2	24	27	23	26	21	20	22	23
Day 2	25	26	23	23	30	23	19	23
Day 28	27	16	12	11	25	17	10	12
Day 90	24	17	13	9	27	17	10	11
Day 181	31	20	13	9	34	20	12	11
Terminal	21	12	9	7	24	13	8	7
Mature B Cells (cells/µL)								
Pretest 1	351	445	367	357	324	231	376	415
Pretest 2	419	432	331	389	269	300	409	450
Day 2	396	243	113	99	325	103	83	81
Day 28	318	60	25	16	239	36	26	23
Day 90	267	53	18	15	261	41	25	22
Day 181	188	30	14	9	223	23	12	19
Terminal	230	20	12	10	210	13	10	9
Mature B Cells (% of gated)								
Pretest 1	17	21	19	19	17	14	20	23
Pretest 2	20	21	19	19	15	15	21	23
Day 2	19	15	10	8	18	9	7	7
Day 28	18	6	3	3	15	4	4	4
Day 90	17	6	3	3	16	3	5	4
Day 181	14	4	3	2	14	3	3	3
Terminal	15	3	2	3	13	2	2	2
Mature T Cells (cells/µL)								

	Females				Males			
Dose(mg/kg/day)	**0**	**2**	**6**	**20**	**0**	**2**	**6**	**20**
Pretest 1	1390	1332	1247	1117	1255	1167	1177	1222
Pretest 2	1232	1412	1119	1380	1277	1140	1157	1251
Day 2	1357	1251	957	1018	1468	1009	923	832
Day 28	1200	775	548	386	1232	827	471	415
Day 90	1210	770	527	409	1348	961	456	384
Day 181	1439	787	562	443	1654	878	450	405
Terminal	947	479	401	314	1250	597	332	320
Mature T Cells (% of gated)								
Pretest 1	73	70	73	70	74	76	70	69
Pretest 2	73	72	72	73	74	73	71	68
Day 2	72	78	77	80	73	78	78	78
Day 28	75	83	79	74	75	82	80	72
Day 90	75	81	79	77	74	78	74	69
Day 181	76	79	76	74	74	75	70	71
Terminal	77	82	83	79	78	82	80	78
CD4+ T Cells (cells/µL)								
Pretest 1	747	842	728	659	755	724	704	699
Pretest 2	680	899	663	823	742	699	705	656
Day 2	748	790	586	624	858	631	550	465
Day 28	685	471	315	216	741	499	261	217
Day 90	678	457	293	210	818	530	238	178
Day 181	820	455	279	219	963	468	221	184
Terminal	523	279	201	151	727	310	160	142
CD4+ T Cells (% of gated)								
Pretest 1	39	44	43	41	44	47	42	39
Pretest 2	40	46	42	43	43	44	43	36
Day 2	39	49	47	49	43	49	47	44
Day 28	42	50	45	41	45	49	45	38
Day 90	42	48	44	40	45	43	39	32
Day 181	43	45	38	36	43	40	35	33
Terminal	43	48	42	38	46	42	39	35
CD8+ T Cells T Cells (cells/µL)								
Pretest 1	489	325	395	337	398	322	333	430
Pretest 2	499	376	342	405	463	415	342	484
Day 2	453	339	269	316	403	290	261	276
Day 28	342	204	128	133	305	191	146	140
Day 90	409	212	162	134	368	358	151	153
Day 181	514	200	206	182	476	313	146	170
Terminal	307	137	122	125	326	208	143	120
CD8+ T Cells (% of gated)								
Pretest 1	27	18	22	21	20	18	19	21

	Females				Males			
Dose(mg/kg/day)	0	2	6	20	0	2	6	20
Pretest 2	25	18	20	20	21	20	18	22
Day 2	25	20	20	22	21	19	23	25
Day 28	24	21	22	20	19	22	24	25
Day 90	23	22	25	27	20	26	24	27
Day 181	24	23	28	31	21	26	28	30
Terminal	25	22	27	33	21	28	31	32

% of gated - % of total leukocytes

Dosing Solution Analysis

The results for venetoclax concentration ranged from 92 to 100% of the nominal concentration for all treatment groups (analyses encompassed the first preparation on Week 1, then monthly intervals, and the last preparation). The results of the homogeneity of dose formulations prepared for Week 1 showed the relative standard deviation of venetoclax concentrations ranged from 0.0 to 0.6% within each treatment group, indicating that the preparations were homogeneous.

7 Genetic Toxicology

7.1 In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: **Salmonella-Escherichia coli Mammalian- Microsome Reverse Mutation Assay with a Confirmatory Assay with A-1195425.**

Study no.:	R&D/10/420
Study report location:	4.2.3.3.1.
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	January 28, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1195425 (venetoclax, A-1195425.0, ABT-199), 1739337, 95.8%[1]

[1]A correction factor value of 1.04 was applied to all formulations to correct for potency.

Key Study Findings

- Venetoclax was negative in tester strains of Salmonella or E.coli in the presence and absence of S-9 mix, under the conditions tested.

Methods

Strains:	Salmonella typhimurium tester strains TA1535, TA1537, TA98, TA100, and E. coli WP2 uvrA

Concentrations in definitive study:	5, 10, 50, 100, 500, 1000, and 5000 µg/plate
Basis of concentration selection:	Levels up to 5000 µg/plate is the standard limit dose recommended by regulatory guidelines
Negative control:	DMSO
Positive control:	With S9: 2-aminoanthracene, benzo[a]pyrene Without S9: sodium azide, ICR-191, 2-nitrofluorene, 4-nitroquinoline N-oxide
Formulation/Vehicle:	Dimethyl sulfoxide (DMSO)
Incubation & sampling time:	Initial mutagenicity assay: plate incorporation Confirmatory mutagenicity assay: Preincubation

Criteria for positive results:
Results were considered positive if the data for any treatment level showed a response ≥ 2 times the concurrent vehicle control for TA98, TA100, WP2 uvrA or ≥ 3 times the concurrent vehicle control for TA1535 and TA1537.

Study Validity:

- Selection of the tester strains was adequate based upon Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICHS2A, April 1996).
- The highest concentration tested was 5000 µg/plate, which allowed maximum exposure.
- A minimum of three non-toxic doses were included for each strain
- The vehicle control values were within the laboratory historical ranges.
- The positive control compounds (± S9 mix) produced increases in the number of revertant colonies.

Results

Plate incorporation assay

Cytotoxicity: >50% reduction in revertant counts was observed with tester strain TA1537 at the top dose with S9.

Precipitate: Venetoclax precipitated from solution at ≥500 µg/plate with all tester strains, with and without S9.

Mutagenicity: Venetoclax was not mutagenic in any of the tester strains with and without S9.

Preincubation assay

Cytotoxicity: No cytotoxicity was observed in any of the tester strains up to 5000 µg/plate.

Precipitate: Venetoclax was insoluble in the aqueous top agar at ≥500 µg/plate with and without S9.

Mutagenicity: A 3.3 fold increase in revertant colonies was observed at 50 µg/plate over the control in the tester strains 1537 without S9. However, this increase was considered

not biologically relevant based on lack of dose response, non-reproducible and below the historical control mean revertants per plate. Venetoclax was not mutagenic in any of the tester strains with and without S9.

Table 43: Initial Bacterial Mutagenicity Assay of Venetoclax – Without S9

(Excerpted from Applicant's NDA)

Metabolic Activation	Test Article	Dose Level (µg/plate)	Assay #1 Revertant Colony Counts (Mean ±SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without Activation	DMSO	50 µl/plate	17 ± 8	85 ± 11	17 ± 3	6 ± 4	16 ± 2
	A-1195425	5.00	14 ± 5	84 ± 8	18 ± 6	5 ± 3	18 ± 6
		10.0	16 ± 0	91 ± 12	16 ± 1	6 ± 2	18 ± 3
		50.0	17 ± 6	88 ± 7	13 ± 5	8 ± 3	19 ± 2
		100	18 ± 2	89 ± 17	16 ± 2	7 ± 4	19 ± 2
		500	17 ± 6[P]	89 ± 5[P]	15 ± 4[P]	6 ± 1[P]	15 ± 5[P]
		1000	15 ± 4[P]	110 ± 16[P]	14 ± 7[P]	4 ± 3[P]	15 ± 2[P]
		5000	10 ± 4[PI]	71 ± 5[PI]	11 ± 1[PI]	4 ± 0[PI]	10 ± 4[PI]
	2-nitrofluorene	1	193 ± 26				
	sodium azide	2		1256 ± 45	982 ± 42		
	ICR-191	2				187 ± 18	
	4-nitroquinoline-N-oxide	1					383 ± 82

Criteria for a positive response (increase over corresponding control): 2-fold for TA98, TA100, and WP2*uvr*A; 3-fold for TA1535 and TA1537)
[P] Precipitation of test article observed; [I] Intensely colored agar

Table 44: Initial Bacterial Mutagenicity Assay of Venetoclax – With S9

(Excerpted from Applicant's NDA)

Metabolic Activation	Test Article	Dose Level (µg/plate)	Assay #1 Revertant Colony Counts (Mean ±SD)				
			TA98	TA100	TA1535	TA1537	WP2*uvr*A
With Activation	DMSO	50 µl/plate	26 ± 1	99 ± 10	13 ± 1	10 ± 4	21 ± 4
	A-1195425	5.00	27 ± 7	114 ± 14	14 ± 4	11 ± 2	23 ± 2
		10.0	23 ± 9	94 ± 7	10 ± 6	8 ± 5	19 ± 3
		50.0	25 ± 6	106 ± 16	11 ± 6	7 ± 5	17 ± 4
		100	27 ± 3	103 ± 6	11 ± 4	10 ± 2	16 ± 3
		500	24 ± 3[P]	109 ± 20[P]	12 ± 5[P]	11 ± 1[P]	20 ± 7[P]
		1000	25 ± 3[P]	101 ± 9[P]	12 ± 4[P]	8 ± 4[P]	15 ± 7[P]
		5000	17 ± 5[PI]	98 ± 10[PI]	10 ± 4[PI]	3 ± 1[PI]	15 ± 2[PI]
	benzo[a]pyrene	2.5	411 ± 57				
	2-aminoanthracene	2.5		1232 ± 279	195 ± 30	127 ± 13	
	2-aminoanthracene	25					414 ± 2

Criteria for a positive response (increase over corresponding control): 2-fold for TA98, TA100, and WP2*uvr*A; 3-fold for TA1535 and TA1537)
[P] Precipitation of test article observed; [I] Intensely colored agar

Table 45: Confirmatory Bacterial Mutagenicity Assay of Venetoclax – Without S9

(Excerpted from Applicant's NDA)

Metabolic Activation	Test Article	Dose Level (µg/plate)	Assay #2 Revertant Colony Counts (Mean ±SD) TA98	TA100	TA1535	TA1537	WP2*uvr*A
Without Activation	DMSO	50 µl/plate	15 ± 2	115 ± 13	15 ± 1	3 ± 1	14 ± 4
	A-1195425	5.00	14 ± 1	84 ± 13	13 ± 3	6 ± 1	14 ± 1
		10.0	14 ± 4	101 ± 16	14 ± 2	3 ± 1	18 ± 1
		50.0	14 ± 2	100 ± 10	11 ± 3	11 ± 3	12 ± 6
		100	17 ± 1	117 ± 10	16 ± 2	3 ± 1	14 ± 6
		500	15 ± 6[P]	105 ± 8[P]	11 ± 3[P]	6 ± 0[P]	15 ± 1[P]
		1000	10 ± 2[P]	90 ± 3[P]	9 ± 1[P]	7 ± 2[P]	14 ± 4[P]
		5000	10 ± 1[PI]	84 ± 8[PI]	10 ± 3[PI]	3 ± 2[PI]	15 ± 4[PI]
	2-nitrofluorene	1	318 ± 37				
	sodium azide	2		1137 ± 83	795 ± 19		
	ICR-191	2				1531 ± 239	
	4-nitroquinoline-N-oxide	0.4					887 ± 36

Criteria for a positive response (increase over corresponding control): 2-fold for TA98, TA100, and WP2*uvr*A; 3-fold for TA1535 and TA1537)
[P] Precipitation of test article observed; [I] Intensely colored agar

Table 46: Confirmatory Bacterial Mutagenicity Assay of Venetoclax – With S9

(Excerpted from Applicant's NDA)

Metabolic Activation	Test Article	Dose Level (µg/plate)	Assay #2 Revertant Colony Counts (Mean ±SD) TA98	TA100	TA1535	TA1537	WP2*uvr*A
With Activation	DMSO	50 µl/plate	20 ± 3	106 ± 4	11 ± 6	6 ± 3	15 ± 4
	A-1195425	5.00	24 ± 4	115 ± 6	10 ± 2	6 ± 3	21 ± 5
		10.0	22 ± 3	98 ± 1	10 ± 4	7 ± 2	17 ± 3
		50.0	23 ± 8	86 ± 17	12 ± 4	7 ± 2	15 ± 3
		100	26 ± 3	106 ± 12	10 ± 4	8 ± 0	19 ± 4
		500	18 ± 7[P]	104 ± 7[P]	12 ± 6[P]	10 ± 3[P]	11 ± 2[P]
		1000	20 ± 5[P]	93 ± 7[P]	11 ± 3[P]	5 ± 1[P]	20 ± 2[P]
		5000	16 ± 5[PI]	94 ± 16[PI]	7 ± 2[PI]	5 ± 2[PI]	9 ± 3[PI]
	benzo[a]pyrene	2.5	426 ± 57				
	2-aminoanthracene	2.5		1551 ± 169	169 ± 11	141 ± 3	
	2-aminoanthracene	25					442 ± 102

Criteria for a positive response (increase over corresponding control): 2-fold for TA98, TA100, and WP2*uvr*A; 3-fold for TA1535 and TA1537)
[P] Precipitation of test article observed; [I] Intensely colored agar

7.2 In Vitro Assays in Mammalian Cells

Study title: **Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes With A-1195425**

Study no.:	R&D/10/421
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	January 28, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1195425 (venetoclax, A-1195425.0, ABT-199), 1739337, 95.8%[1]

[1]A correction factor value of 1.04 was applied to all formulations to correct for potency.

Key Study Findings

- Venetoclax was negative for the induction of structural or numerical aberrations in the in vitro human peripheral lymphocyte culture in the presence and absence of S-9 mix, under the conditions tested.

Methods

Cell line:	Human peripheral whole blood lymphocytes
Concentrations in definitive study:	6, 9, 12.2, 17.5, 20.7, 24, 26.5, 29.8, 35, and 50 µg/mL without S9 (3-hour treatment); 4, 6, 9, 12.2, 15, 17.6, 20.7, 24, 26.5, 32, and 40 µg/mL without S9 (~22-hour treatment); 9, 12.2, 17.5, 26.5, 32, 35, 40, 45, 50, and 70 µg/mL with S9 (3-hour treatment) were tested.
Basis of concentration selection:	Based on range finding study at concentrations of A-1195425 ranging from 1.17 to 1200 µg/mL with and without S9.
Negative control:	DMSO
Positive control:	Mitomycin C (MMC) without S9 Cyclophosphamide (CP) with S9
Formulation/Vehicle:	DMSO
Incubation & sampling time:	3 hours with or without S9 and harvest at ~22 hours; ~22 hours without S9 and harvest at 22 hours

Criteria for positive results:
A test article will be considered positive for inducing chromosomal aberrations if a significant increase (the difference will be considered significant when $p \leq 0.01$) in the number of cells with chromosomal aberrations is observed at one or more dose levels. The linear trend test evaluates the dose-responsiveness. If a significant increase is seen at one or more dose levels, a dose-response should be observed.

Study Validity
The positive control result was significantly higher ($p \leq 0.01$) than the vehicle control. The highest dose selected to analyze chromosome aberrations was justified for the 3 hour and 22 hour treatment in the absence of S9 to have (~≥50%); however, the high dose selected for chromosome analysis in the S9 3 hour treatment group did not meet the 50% reduction criteria. There were three analyzable dose levels in each treatment condition.

Reviewer's comment: An Information Request was sent to the Applicant to address the following: In the 3 hour S9-activated treatment group, the high dose selected (50 µg/mL) for chromosome aberrations analysis did not meet the acceptance criteria required to justify the high dose (page16 of the report).

The Applicant's response: An amended final report was submitted: Revised page 20 of the amended final report addressed FDA concern: When analysis for aberrations was performed, 100 cells, if possible, from each replicate culture from three concentrations

of the test article, vehicle controls, and one dose of the positive control cultures were analyzed for the different types of chromosomal aberrations (Evans, 1962, 1976) with the exception that 200 cells were analyzed from culture A only, at the high dose, with a 3-hour treatment in the presence of S9. Culture A of the 50 µg/mL dose level had a 51% reduction in mitotic index compared to the average MI of the vehicle control cultures. While the average MI reduction of culture A and B at 50 µg/mL was only 43%, in order to evaluate at the appropriate target limiting toxicity (≥50 % MI reduction) only culture A was used and all cells evaluated from the 50 µg/mL dose with S9 were from culture A.

Reviewer's comment: The Applicant's response is acceptable.

Results

Cytotoxicity: High dose to analyze for chromosome aberrations was selected based >50% reduction in mitotic index compared to respective DMSO control groups in the 3 hour and the 20 hour non-activation system; The high dose selected for chromosome aberration analysis in the 3 hour S9-activated system had 43% reduction in mitotic index.

Clastogenicity: No statistically significant increase in structural or numerical (endoreduplication or polyploidy) chromosome aberrations were observed up to >50% cytotoxic dose with or without S9.

Table 47: Summary of Chromosome Aberrations Study of Venetoclax–Without S9

(Excerpted from Applicant's NDA)

Metabolic Activation	Test Article	Concentration	Cytotoxicity (% of control)[a]	% Aberrant Cells Mean -g	% Aberrant Cells Mean +g	Aberrations per Cell[b]	% Poly-ploid	% Endo-reduplicate
3 hr Without Activation	Vehicle (DMSO)	10.0 µL/mL	100	0.0	2.0	0.0	0.0	0.0
	A-1195425	24.0 µg/mL	100	0.0	0.5	0.0	1.0	0.0
		29.8 µg/mL	70	0.0	1.0	0.0	0.5	0.0
		50.0 µg/mL	49	0.0	1.5	0.0	1.0	0.0
	Mitomycin C	1.00 µg/mL[c]	--	32.8*	36.0	0.46	0.0	0.0
22 hr Without Activation	Vehicle (DMSO)	10.0 µL/mL	100	0.5	0.5	0.01	0.0	0.0
	A-1195425	12.2 µg/mL	79	0.0	1.5	0.0	0.0	0.0
		17.6 µg/mL	61	0.0	1.0	0.0	0.0	0.0
		20.7 µg/mL	49	0.0	1.5	0.0	0.5	0.0
	Mitomycin C	0.300 µg/mL[d]	--	41.0*	48.0	0.58	0.0	0.0

Table 48: Summary of Chromosome Aberrations Study of Venetoclax–With S9

(Excerpted from Applicant's NDA)

Metabolic Activation	Test Article	Concentration	Cytotoxicity (% of control)[a]	% Aberrant Cells Mean -g	% Aberrant Cells Mean +g	Aberrations per Cell[b]	% Poly-ploid	% Endo-reduplicate
3 hr With Activation	Vehicle (DMSO)	10.0 µL/mL	100	1.5	2.5	0.02	0.0	0.0
	A-1195425	40.0 µg/mL	100	2.0	2.5	0.04	0.0	0.0
		45.0 µg/mL	93	1.5	2.0	0.02	1.0	1.0
		50.0 µg/mL[c]	49[c]	3.5	4.0	0.06	0.0	0.0
	Cyclophosphamide	25.0 µg/mL[d]	--	35.0*	39.0	0.49	0.5	0.0

* Significantly greater in -g than the vehicle control, $p \leq 0.01$. Statistical analysis employed a Cochran-Armitage test for linear trend and Fisher's Exact Test to compare the percentage of cells with aberrations (excluding gaps) in treated cells to the results obtained for the vehicle controls.

a – Based on mitotic indices; at least 1000 cells/culture analyzed.

b – Cell which contained >4 aberrations (mab) were calculated as having 5 aberrations; ≥ is used in these instances since the exact number of aberrations was not recorded.

c – 75 cells analyzed from the A culture and 50 cells analyzed from the B culture.

d – 50 cells analyzed from the A culture and 50 cells analyzed from the B culture.

e – Only cells from culture A were analyzed for chromosomal aberrations. The % mitotic index reduction for culture A (contributing 200 cells for chromosome aberration) was 51% compared to the average % mitotic index of the vehicle cultures (contributing 100 cells each for chromosome aberration evaluation).

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: **In Vivo Mouse Bone Marrow Micronucleus Assay with A-1195425** (b) (4)

Study no:	R&D/12/675
Study report location:	4.2.3.3.2.
Conducting laboratory and location:	(b) (4) (b) (4).
Date of study initiation:	July 8, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1195425 (venetoclax, ABT-199, A-1195425.0; (b) (4) 97059-1, 99.1%

Formulations were prepared to compensate for potency using a factor of 8.410.

Key Study Findings

- A-1195425 was negative in the mouse bone marrow micronucleus assay under the conditions of this assay.

Methods

Doses in definitive study:	Vehicle, placebo, 208.8, 417.5, and 835 mg/kg A-1195425
Frequency of dosing:	Single dose administration
Route of administration:	Oral gavage
Dose volume:	20 mL/kg
Formulation/Vehicle:	Reverse osmosis (RO) water and 0.1% (v/v) Antifoam C Emulsion (A-1195425 vehicle)
Species/Strain:	Hsd:ICR(CD-1) mice
Number/Sex/Group:	5/sex/dose group/time point
Satellite groups:	3/sex for vehicle and placebo and 18/sex of each test article concentration
Basis of dose selection:	Range finding study at 833.4 mg/kg (highest

feasible dose) to 6 males and 6 females. No test article-related clinical observations were seen

Negative control: VENETOCLAX Placebo [redacted]
Positive control: Cyclophosphamide

* The formulation consists of [redacted]

Dosing Scheme for the Micronucleus Assay

Group Number	Target Dose Level (mg/kg)	Stock Concen-tration (mg/mL)	Dosing Volume (mL/kg)	Route of Admin-istration	Animals/Harvest Timepoint 24 Hour		48 Hour	
					Male	Female	Male	Female
1/1	Positive Control, 80	8	10	Oral Gavage	5	5	-	-
2/1	Placebo Control, 0	0	20	Oral Gavage	5	5	5	5
3/1	Vehicle Control, 0	0	20	Oral Gavage	5	5	5	5
4/1	A-1195425, 208.8	10.44	20	Oral Gavage	5	5	-	-
5/1	A-1195425, 417.5	20.875	20	Oral Gavage	5	5	-	-
6/1	A-1195425, 835	41.75	20	Oral Gavage	5	5	5	5

Vehicle Control = reverse osmosis (RO) water and 0.1% (v/v) Antifoam C Emulsion (Sigma #A8011)
Positive Control = Cyclophosphamide

Dosing Scheme for the Additional Toxicokinetic Study

Group Number	Target Dose Level (mg/kg)	Stock Concentration (mg/mL)	Dosing Volume (mL/kg)	Route of Administration	Toxicokinetic Animals	
					Male	Female
2/2	Placebo Control, 0	0	20	Oral Gavage	3	3
3/2	Vehicle Control, 0	0	20	Oral Gavage	3	3
4/2	A-1195425, 208.8	10.44	20	Oral Gavage	18	18
5/2	A-1195425, 417.5	20.875	20	Oral Gavage	18	18
6/2	A-1195425, 835	41.75	20	Oral Gavage	18	18

Vehicle Control = reverse osmosis (RO) water and 0.1% (v/v) Antifoam C Emulsion (Sigma #A8011)
a Three mice per sex per timepoint per group were bled for possible measurement of plasma levels at approximately 0.5, 1, 3, 6, 12, and 24 hours after dosing. Each animal was only be bled once. In addition, three mice per sex were dosed with the vehicle control and three mice per sex were dosed with placebo control and bled approximately 1 hour postdose.

Study Validity

Assay Acceptance Criteria

The individual animals in the vehicle control group had less than the historical vehicle control value of approximately 0.4% micronucleated PCE and the group mean was within the current historical control range for males and females. The positive control

group had a statistically significantly higher ($p < 0.01$) number of micronucleated PCEs than the vehicle control group and was consistent with historical positive control data.

Acceptable High Dose
The high dose was 835 mg/kg, (b) (4)

Positive Response: A statistically significant increase in micronucleated PCEs for at least one dose level, and a statistically significant dose-related response at either sampling time, along with a biological interpretation.

Results
Toxicity: Venetoclax was cytotoxic to the bone marrow (i.e., statistically significant decreases in the PCE:NCE ratios) at 417.5 and 835 mg/kg in male animals and 835 mg/kg in female animals.
Clastogenicity: A statistically significant increase in micronucleated PCEs was observed in females at high dose in the 24 hour sacrifice time. However, this increase was not considered to be biologically significant since the percent micronucleated PCEs from the high dose females (0.14%) were within historical control values which range from 0.00 to 0.15%.

Table 49: Summary of In Vivo Mouse Micronucleus Assay with Venetoclax

(Excerpted from Applicant's NDA)

Study No.: 8266991
Test Article: A-1195425
Initiation of Dosing: 31 July 2012 (Males) and 12 September 2012 (Females)
Species/Strain: Mouse/CD-1

Treatment	Dose	Harvest Time	% Micronucleated PCEs Mean ± SD		Ratio PCE:NCE Mean ± SD	
			Male	Female	Male	Female
Controls						
Vehicle	20 mL/kg	24	0.02 ± 0.03	0.03 ± 0.03	0.79 ± 0.21	0.63 ± 0.06
		48	0.07 ± 0.06	0.03 ± 0.04	0.65 ± 0.18	0.79 ± 0.15
Placebo	0 mg/kg (298 mg/mL)	24	0.03 ± 0.04	0.03 ± 0.04	0.58 ± 0.21	0.92 ± 0.13**
		48	0.01 ± 0.02	0.02 ± 0.03	0.58 ± 0.31	0.89 ± 0.10
Positive[a]	CP 80 mg/kg	24	2.55 ± 0.56*	1.61 ± 0.39*	0.49 ± 0.16**	0.68 ± 0.10
Test Article	208.8 mg/kg	24	0.07 ± 0.04	0.07 ± 0.06	0.58 ± 0.24	0.57 ± 0.26
	417.5 mg/kg	24	0.01 ± 0.02	0.07 ± 0.06	0.28 ± 0.14**	0.41 ± 0.23
	835 mg/kg	24	0.02 ± 0.03	0.14 ± 0.08**	0.16 ± 0.13**	0.33 ± 0.07**
		48	0.04 ± 0.04	0.10 ± 0.09	0.63 ± 0.24	0.74 ± 0.14

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
** Significantly different from the corresponding vehicle control, $p \leq 0.05$.
[a] Animal A00947 was not included in statistical analysis.
Vehicle = Reverse Osmosis Water and 0.1% (v/v) Antifoam C Emulsion
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

Historical control data
Mouse Micronucleus - 1/2010 through 11/2010

		% Micronucleated PCEs		PCE:NCE Ratio	
		Males	Females*	Males	Females*
Pooled Vehicle Controls					
24 hour harvest	Minimum	0.00	0.00	0.21	0.35
	Maximum	0.10	0.15	0.86	0.81
	Mean ± SD	0.042 ± 0.038	0.080 ± 0.067	0.511 ± 0.134	0.549 ± 0.211
	N	55	5	55	5
48 hour harvest	Minimum	0.00	0.00	0.25	0.46
	Maximum	0.20	0.20	0.76	1.54
	Mean ± SD	0.047 ± 0.050	0.060 ± 0.057	0.530 ± 0.136	0.740 ± 0.316
	N	50	10	50	10
Positive Controls – Cyclophosphamide					
24 hour harvest	Minimum	0.45	0.70	0.16	0.44
	Maximum	3.65	5.05	0.93	0.55
	Mean ± SD	2.004 ± 0.860	2.380 ± 1.633	0.543 ± 0.170	0.486 ± 0.042
	N	55	5	55	5

PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte
N = Number of animals
SD = Standard deviation
* Pooled vehicle control 48 hour harvest female data from 1/2009 to 12/2009.

7.4 Other Genetic Toxicity Studies

The genetic toxicity potential of the major human metabolite, M27, was tested in GLP compliant in vitro Ames and chromosomal aberration assays. Other impurities with structural alerts were also tested in a bacterial mutation assay, in vitro chromosomal aberration assay and a four week mouse study.

Study title: Bacterial Reverse Mutation Assay in 6-Well Plates with A-1621332 (b) (4)

Study no.:	R&D/14/1122
Study report location:	4.2.3.7.5.
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	September 11, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1621332.0 ((b) (4) M27 metabolite of VENETOCLAX, Lot# 2207614, purity 92.3%[1]

[1] Dosing formulations were adjusted to compensate for the purity of the test article (92.3%), using a correction factor of 1.08.

Key Study Findings

- A-1621332 was not mutagenic in tester strains of Salmonella or E.coli in the presence and absence of S-9 mix in 6-well plates, under the conditions tested.

Methods

Strains:	Salmonella typhimurium tester strains TA98, TA100, TA1535 and TA97a and Escherichia coli tester strain WP2 uvrA
Concentrations in definitive study:	0.30, 1.0, 3.0, 10, 30, 100, 300 and 1000 µg per well
Basis of concentration selection:	1000 µg per well
Negative control:	DMSO
Positive control:	With S9: 2-aminoanthracene, Without S9: sodium azide, ICR-191, 2-Nitrofluorene[1]
Formulation/Vehicle:	Dimethyl sulfoxide (DMSO)
Incubation & sampling time:	Modified plate incorporation method: 48 to 72 hours at 37±2°C

[1] Positive controls were plated concurrently with the plate incorporation mutagenicity assay.

Criteria for positive results:
Results were considered positive if the data for any treatment level showed a concentration-related increase in the mean revertants per well of at least one tester strain over a minimum of two increasing concentrations of test article (≥3x increase for 1535 and ≥2x increase for TA98, TA100, TA97a and WP2 uvrA the mean control value with a minimum 6 revertants).

Study Validity:
- Selection of the tester strains was adequate based upon Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICHS2A, April 1996).
- A minimum of three non-toxic doses were included for each strain.
- Tester strain culture titers were greater than or equal to $0.3x10^9$ cells/mL.
- The vehicle control values were within the laboratory historical ranges.
- The positive control compounds (± S9 mix) produced increases in the number of revertant colonies (at least 3x increase in the number of revertants over the mean value of the respective negative control).

Results
- No background lawn toxicity was observed.
- Precipitate was observed beginning at 100 or 300 µg per well.
- No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation.

Table 50: Bacterial Mutagenicity Assay with A-1621332.0 ((b) (4) M27 Metabolite

(Excerpted from Applicant's NDA)

Test Article Name: A-1621332 Experiment Number: B1
Study Number: AE03RW.502008.BTL

Concentration (µg/well)	TA98	TA100	TA1535	TA97a	WP2 uvrA
Average Revertants Per Well ± Standard Deviation Without Activation					
DMSO	4 ± 2	24 ± 1	7 ± 1	22 ± 1	7 ± 1
0.3	4 ± 3	17 ± 6	3 ± 2 d	18 ± 2	3 ± 1 d
1.0	5 ± 2	18 ± 1	4 ± 1	20 ± 3	3 ± 2 d
3.0	4 ± 1	23 ± 3	4 ± 2	21 ± 7	5 ± 1
10	6 ± 2	19 ± 1	4 ± 2	21 ± 6	7 ± 1
30	4 ± 2	16 ± 2	3 ± 1 d	19 ± 3	6 ± 4
100	4 ± 2	16 ± 3	3 ± 2 d	17 ± 2 b b b b	6 ± 3 b b b b
300	4 ± 2 b b b b	23 ± 9 b b b b	2 ± 2 b d b b	19 ± 2 b b b b	5 ± 2 b b b b
1000	5 ± 4 b b	26 ± 1 b b	3 ± 1 b d b	16 ± 4 b b	4 ± 3 b b
Positive Control					
2-Nitrofluorene	24 ± 3 ##				
Sodium Azide		171 ± 14 ##	142 ± 25 ##		
ICR-191				483 ± 62 ##	
Methylmethane sulfonate					71 ± 10 ##
Average Revertants Per Well ± Standard Deviation With Activation					
DMSO	6 ± 1	20 ± 3	2 ± 1	20 ± 6	7 ± 3
0.3	6 ± 1	21 ± 12	3 ± 1	24 ± 5	9 ± 2
1.0	6 ± 2	23 ± 3	5 ± 2	17 ± 4	5 ± 1
3.0	5 ± 2	17 ± 5	3 ± 1	19 ± 4	7 ± 2
10	7 ± 4	20 ± 3	3 ± 1	22 ± 9	5 ± 3
30	6 ± 1	16 ± 2	4 ± 2	23 ± 7	9 ± 2
100	7 ± 3	21 ± 4	4 ± 2	20 ± 7 b b b b	5 ± 3 b b b b
300	7 ± 1 b b b b	20 ± 5 b b b b	2 ± 1 b b b b	19 ± 3 b b b b	9 ± 2 b b b b
1000	4 ± 2 b b	28 ± 6 b b	5 ± 1 b b	16 ± 4 b b	4 ± 2 b b
Positive Control					
2-Aminoanthracene	168 ± 18 ##	193 ± 27 ##	31 ± 4 ##	180 ± 8 ##	83 ± 15 ##

a = Cytotoxic concentration (reduced background lawn); b = Precipitate present; c = obscured by precipitate; d = reduction in revertant count
T = Too numerous to count; NA = Not applicable; Untreated = Untreated Control
@ = greater than or equal to 2.0 times vehicle control; ## = greater than or equal to 3.0 times the vehicle control

In Vitro Assays in Mammalian Cells

Study title: In Vitro Mammalian Chromosome Aberration Assay in Human Peripheral Blood Lymphocytes (HPBL) with A-1621332 (b) (4)

Study no.:	R&D/14/1123
Study report location:	4.2.3.7.5
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	August 19, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1621332.0 ((b) (4) M27 metabolite of VENETOCLAX Lot # 2207614; Purity 92.3%[1]

[1] Dosing formulations were adjusted to compensate for the purity of the test article (92.3%), using a correction factor of 1.08.

Key Study Findings

- A-1621332 was negative for the induction of structural or numerical aberrations in the in vitro human peripheral lymphocyte culture in the presence and absence of S-9 mix, under the conditions tested.

Methods

Cell line:	Human peripheral whole blood lymphocytes
Concentrations in definitive study:	2.5, 5, 10, 12.5, 15, 17.5, and 20 µg/mL without S9 (3-hour treatment); 1, 2.5, 5, 7.5, 10, 12.5, and 15 µg/mL without S9 (~22-hour treatment and with S9 (3-hour treatment) were tested.
Basis of concentration selection:	Based on range finding study at concentrations ranging from 0.05 to 500 µg/mL with and without S9.
Negative control:	DMSO
Positive control:	Mitomycin C (MMC) without S9 Cyclophosphamide (CP) with S9
Formulation/Vehicle:	DMSO
Incubation & sampling time:	3 hours with or without S9 and harvest at ~22 hours; ~22 hours without S9 and harvest at 22 hours

Criteria for positive results:

A test article will be considered positive for inducing chromosomal aberrations if a significant increase (the difference will be considered significant when $p \leq 0.01$) in the number of cells with chromosomal aberrations is observed at one or more dose levels. The linear trend test evaluates the dose-responsiveness. If a significant increase is seen at one or more dose levels, a dose-response should be observed.

Study Validity
The positive control result was significantly higher ($p \leq 0.01$) than the vehicle control. The highest dose selected to analyze chromosome aberrations was justified. There were three analyzable dose levels in each treatment condition.

Results
Cytotoxicity: High dose to analyze for chromosome aberrations was selected based >50% reduction in mitotic index compared to respective DMSO control groups in all 3 treatment conditions.
Clastogenicity: No statistically significant increase in structural or numerical (endoreduplication or polyploidy) chromosome aberrations were observed up to >50% cytotoxic dose with or without S9.

Table 51: Summary of In Vitro Chromosome Aberration Assay in HPBL with A-1621332 (b) (4) (M27) Metabolite

(Excerpted from Applicant's NDA)

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Structural (%)
				Numerical	Structural				
DMSO	-S9	4	18.3	200	200	0.000	±0.000	0.0	0.0
A-1621332									
5	-S9	4	18.1	200	200	0.000	±0.000	0.0	0.0
10	-S9	4	16.8	200	200	0.000	±0.000	0.0	0.0
17.5	-S9	4	8.3	200	200	0.000	±0.000	0.0	0.0
MMC, 0.6	-S9	4	14.3	200	100	0.210	±0.456	0.0	19.0**
DMSO	+S9	4	16.1	200	200	0.005	±0.071	0.0	0.5
A-1621332									
2.5	+S9	4	16.0	200	200	0.000	±0.000	0.0	0.0
5	+S9	4	11.0	200	200	0.000	±0.000	0.0	0.0
7.5	+S9	4	7.2	200	200	0.005	±0.071	0.0	0.5
CP, 5	+S9	4	6.9	200	100	0.180	±0.411	0.0	17.0**
DMSO	-S9	20	18.9	200	200	0.005	±0.071	0.0	0.5
A-1621332									
2.5	-S9	20	17.6	200	200	0.000	±0.000	0.0	0.0
5	-S9	20	15.3	200	200	0.000	±0.000	0.0	0.0
7.5	-S9	20	8.4	200	200	0.000	±0.000	0.0	0.0
MMC, 0.3	-S9	20	10.6	200	100	0.250	±0.500	0.0	23.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using the Fisher's Exact test.

Following development of the commercial synthetic route for production of venetoclax drug substance, 4 drug substance impurities and 2 drug product degradants were identified in venetoclax that were not qualified in previous toxicology studies in animals. These impurities and degradants were assessed in a GLP 4-week mouse repeat-dose toxicology study and by GLP in vitro and in vivo genotoxicity assays.

Study title: Four-Week Oral (Gavage) Toxicity Study of A-1195425 (with Impurities) in CD-1 Mice

Study no.:	R&D/13/545
Study report location:	4.2.3.7.6.
Conducting laboratory and location:	AbbVie Deutschland GmbH & Co. KG Germany
Date of study initiation:	June 4, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	1: A-1195425 (venetoclax, A-1195425.0, A-1195425 (b) (4) ABT-199), sp (b) (4) d with four impurities at approximately % each and approximately (b) (4) % of the (b) (4) degradant and approximately (b) (4) % of the (b) (4) degradant based on A-1195425 content. Lot# 10027244-0102, purity 101.0% Impurities/Degradants (%w/w of A-1195425): (b) (4) API impurity-1 API impurity-2 API impurity-3 API impurity-4 DP impurity- (b) (4) DP impurity- (b) (4) 2: A-1195425 (venetoclax, A-1195425.0, A-1195425 (b) (4) ABT-199), Lot # 10027244-0104, purity 100.3%

Key Study Findings

- Targets organs of toxicity include, lymphatic system and red cell mass.
- There were no additional toxicities related to the impurities.

Methods

Doses: 0, 250 A-1195425 + spiked impurities[1], and 250 A-1195425 mg/kg[2]

Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	20 mL/kg
Formulation/Vehicle:	Tween 80:Copovidone:Colloidal SiO2 ((b) (4) % by weight); placebo
Species/Strain:	CD-1[CD® (SD)] mice
Number/Sex/Group:	10 per sex/group
Age:	Approximately 11 weeks
Weight:	Males: 26.2 to 37.9 g; Females: 22.5 to 29.1 g
Satellite groups:	

[1] A-1195425.0:Tween 80:Copovidone:Colloidal SiO2 ((b) (4) % by weight with (b) (4) % added impurities and degradants
[2] A-1195425.0:Tween 80:Copovidone:Colloidal SiO2 (b) (4) %, by weight)

Test Group	Color Code	Dosage A-1195425 (mg/kg/day)[a]	Concentration of A-1195425 (mg/mL)[e]	Number of Mice	
				Male	Female
Group 1 (control item; placebo)	white	0[b]	0	10	10
Group 2 (A-1195425; reference item;not spiked)	blue	250[c]	12.5	10	10
Group 3 (A-1195425; test item; spiked with degradants/impurities)	green	250[d]	12.5	10	10

a. Expressed as mg free active moiety/mL.
b. Control Item: Tween 80:Copovidone:Colloidal SiO_2 ((b) (4) %, by weight); suspended in water for injection for dosing.
c. A-1195425 (b) (4) no added impurities/degradants). Assigned chemical potency: 120.3 mg A-1195425.0/g (b) (4) (100.3% of theory), Lot No. 10027244-0104. Suspended in water for injection for dosing.
d. A-1195425 (b) (4) with spiked impurities and degradants). Assigned chemical potency: 121.2 mg A-1195425.0/g (b) (4) (101.0% of theory), Lot No. 10027244-0102. Suspended in water for injection for dosing.
e. The dose volume was 20 mL/kg.

Clinical Findings	Twice Weekly
Body weights	Twice Weekly
Food consumption	Weekly
Hematology	Prior to the terminal necropsy
Clinical chemistry	Prior to the terminal necropsy
Gross pathology	Terminal necropsy
Organ weights	Terminal necropsy
Histopathology	Terminal necropsy

Mortality
All animals survived to the scheduled necropsy

Clinical Signs
Unremarkable

Body Weights
Unremarkable

Food Consumption
Unremarkable

Hematology

Table 52: Mean % Change in Hematology Parameters of Venetoclax With or Without Spiked Impurities Compared to Control in a 4-week Repeat Dose Study in Mice

Grp	WBC	LYM	MON	EOS	BAS	RBC	HgB	Hct	Mcv	MCH	RDW	Rct	PLT
	E9/L	E9/L	E9/L	E9/L	E9/L	E12/L	g/dL	%	fL	pg	%	E9/L	E9/L
Males													
2	-65	-76	-39	-84	-20	-4	-15	-12	-8	-11	15	21	21
3	-54	-65	-29	-82	-20	0	-12	-8	-8	-12	16	20	16
Females													
2	-73	-81	-73	-38	-100	0	-10	-8	-9	-11	14	16	2
3	-71	-80	-69	-61	-43	-1	-12	-8	-7	-11	17	24	8

Clinical Chemistry
Unremarkable

Gross Pathology
Unremarkable

Organ Weights

Histopathology
Adequate Battery
Yes.

Peer Review
Yes.

Histological Findings

- Minimal to mild (males) and minimal to moderate (females) increases of splenic extramedullary hematopoiesis were present in all animals dosed with 250 mg/kg/day A-1195425 with and without impurities.
- Minimal to mild decreases of lymphocytes in the spleen were seen in two males and eight females dosed with A-1195425 without impurities and in three males and seven females dosed with A-1195425 with impurities.

8 Carcinogenicity

Not conducted for this indication.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Study of Fertility and Early Embryonic Development to Implantation of A-1195425 (b) (4) Administered by Oral Gavage in Male Mice

Study no.:	R&D/12/810 (Sponsor Reference No. TD12-159)
Study report location:	4.2.3.5.1.
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	August 21, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1195425 (venetoclax, A- 195425.0 (b) (4), ABT-199 (b) (4) or A-1195425.0), 97059-1, 99.1%.

Text Table 1
Experimental Design

Group No.	Test Material	A-1195425 Dose Level (mg/kg/day)	A-1195425 Concentration (mg/mL)[a]	Dose Volume (mL/kg)	Number of Male Mice	Number of Female Mice
1	Control Article	0	0	20	25	25
2	A-1195425	50	2.5	20	25	25
3	A-1195425	200	10	20	25	25
4	A-1195425	600	30	20	25	25

[a] The test article A-1195425, suspended in Sterile Water for Injection (SWFI), USP; assigned chemical potency = 118.9 mg A-1195425.0/gram (b) (4) formulation concentrations was adjusted for assigned chemical potency (correction factor = 8.41). The control article, Placebo Milled Extrudate, was suspended in SWFI, USP, and administered in an amount equal to that given at the high dose; correction factor = 7.41.

Key Study Findings

- All doses were well tolerated by male mice.
- There are no toxic effects of venetoclax on paternal toxicity, male mating and fertility and early embryonic development up to 600 mg/kg/day, the highest dose tested.

Methods

Doses:	0, 50, 200, and 600 mg/kg
Frequency of dosing:	Daily
Dose volume:	20 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Placebo (b) (4)
Species/Strain:	Crl:CD1(ICR) mice
Number/Sex/Group:	25
Study design:	Male mice were administered test or control

article 14 days prior to cohabitation, during cohabitation and continuing through the day before euthanasia.

Treated Male mice:

Mortality
No venetoclax-related deaths at any dose.

One male (no. 3492) at 600 mg/kg/day, was found dead on Day 29 and another male (no. 3483) in this group was euthanized on Day 38 because of clinical signs associated with a gavage accident.

Clinical Signs
Dehydration (n= 14) and hunched posture (N=3) at 600 mg/kg/day compared to controls.

Body Weight
Unremarkable

Mating
Unremarkable

Fertility
Unremarkable

Reproductive organ weights
Unremarkable

Necropsy
Unremarkable

Untreated female mice:

Mortality
No deaths occurred in female mice.

Clinical Signs
Unremarkable

Body Weight
Unremarkable

Necropsy
Unremarkable

Ovarian and uterine examinations
Unremarkable

Study title: Study of Fertility and Early Embryonic Development to Implantation of A-1195425 [redacted] Administered by Oral Gavage in Female Mice

Study no.: R&D/13/279 (Sponsor Reference No. TD12-050)
Study report location: 4.2.3.5.1.
Conducting laboratory and location: [redacted]
Date of study initiation: August 14, 2012
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: A-1195425 (venetoclax, A- 195425.0 [redacted], ABT-199 [redacted] or A-1195425.0), 97059-1, 99.1%.

Experimental Design

Group No.	Test Material	A-1195425 Dose Level (mg/kg)	A-1195425 Concentration (mg/mL)[a]	Dose Volume (mL/kg)	Number of Female Mice
1	Control Article	0	0	20	25
2	A-1195425	50	2.5	20	25
3	A-1195425	200	10	20	25
4	A-1195425	600	30	20	25

[a] The test article A-1195425, suspended in Sterile Water for Injection (SWFI), USP; assigned chemical potency = 118.9 mg A-1195425.0/gram [redacted] formulation concentrations was adjusted for assigned chemical potency (correction factor = 8.41). The control article, Placebo [redacted] was suspended in SWFI, USP, and administered in an amount equal to that given at the high dose; correction factor = 7.41.

Key Study Findings

- All doses were well tolerated by female mice.
- There are no toxic effects of venetoclax on maternal toxicity, estrous cycling or any mating and fertility parameter.
- All ovarian and uterine parameters were comparable among all dose groups including controls.

Methods

Doses: 0, 50, 200, and 600 mg/kg
Frequency of dosing: Daily
Dose volume: 20 mL/kg
Route of administration: Oral gavage
Formulation/Vehicle: Placebo [redacted]
Species/Strain: Crl:CD1(ICR) mice
Number/Sex/Group: 25
Study design: Female mice were administered test or control article orally via gavage once daily beginning 15 days before cohabitation, during cohabitation and continuing until gestation day (GD) 7 at doses of 0, 50, 200 and 600 mg/kg/day. Treated females underwent up to 21 days of cohabitation

with untreated males. Caesarean sections were performed on Day 13 of gestation.

Terminal Procedures – Female Mice

Group No.	No. of Female Mice	Scheduled Euthanasia Day	Necropsy Procedures				Histology	Histopathology
			Ovarian/ Uterine Examination	Necropsy	Tissue Collection	Organ Weights		
1	25	GD 13	X	X	X[a]	-	-	-
2	25						-	-
3	25						-	-
4	25						-	-
Unscheduled Deaths[b]			Pregnancy Status	X	X[a]	-	-	-

X = procedure to be conducted; - = not applicable.
[a] See Tissue Collection and Preservation table for listing of tissues.
[b] See Section 15.2. (Unscheduled Deaths).

Mortality
No venetoclax-related deaths at any dose.

One female in each of the 50 (animal # 5034) and 600 mg/kg (animal # 5097) dose groups were euthanized on DS 15 or DS 16 because of clinical signs associated with a gavage accident including perforation in the tongue and trachea proximal to the bifurcation of the lungs in animal #5034 and two perforations in the esophagus in animal # 5097).

Clinical Signs
Unremarkable

Body Weight
Unremarkable

Estrous cycling,
Unremarkable

Mating
Unremarkable

Fertility,
Unremarkable

Ovarian and Uterine examinations
Unremarkable

Gross necropsy
Unremarkable

9.2 Embryonic Fetal Development

Study title: An Embryo-fetal Development Study of A-1195425 (b) (4) by Oral Gavage in Mice

Study no.:	R&D/12/746 (Sponsor Reference No. TD12-053)
Study report location:	4.2.3.5.2.
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	August 7, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1195425 (venetoclax, A- 195425.0 (b) (4), ABT-199 (b) (4) or A-1195425.0), 97059-1, 99.1%.

Key Study Findings:

- A decrease in the number of implantations, and live fetuses, and an increase in post-implantation loss were noted at 150 mg/kg/day.
- Decrease in male fetal body weights was observed at the high dose 150 mg/kg.

Text Table 3
Experimental Design

Group No.	Test Material	A-1195425 Dose Level (mg/kg/day)	A-1195425 Concentration (mg/mL)[a]	Dose Volume (mL/kg)	No. of Main Study Mice (Assigned Mouse Numbers)	No. of Toxicokinetic Mice (Assigned Mouse Numbers)[b]
1	Control Article	0	0	20	25 (2801 -2825)	6 (2901 - 2906)
2	A-1195425	10	0.5	20	25 (2826 - 2850)	30 (2907 - 2936)
3	A-1195425	50	2.5	20	25 (2851 - 2875)	30 (2937 - 2966)
4	A-1195425	150	7.5	20	25 (2876 - 2900)	30 (2967 - 2996)

[a] The test article A-1195425, suspended in Sterile Water for Injection (SWFI), USP; assigned chemical potency = 118.9 mg A-1195425.0/gram (b) (4) formulation concentrations were adjusted for assigned chemical potency (correction factor = 8.41). The control article, Placebo (b) (4) was suspended in SWFI, USP, and administered in an amount equal to that given at the high dose; correction factor = 7.41.

[b] Toxicokinetic mice were used for blood collection purposes only.

Methods

Doses:	0, 10, 50, and 150 mg/kg
Frequency of dosing:	once daily on gestation days (GD) 6 through 15
Dose volume:	20 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	(b) (4) suspended in Sterile Water for Injection, USP
Species/Strain:	Crl:CD1(ICR) pregnant female mice
Number/Sex/Group:	25 female mice for EFD study
Satellite groups:	30 female mice for each test article dose for TK study; 6 female mice in the control TK study
Study design:	The control article or the test article was administered orally via gavage once daily on gestation days (GD) 6 through 15. All mice

assigned to main study were euthanized on GD 18.

Observations and Results

Mortality
There was no A-1195425-related mortality at any dose. Two control mice were euthanatized due to early delivery.

Clinical Signs
Unremarkable

Body Weight
Mice in the 150 mg/kg dose group exhibited a decrease in maternal body weight gain, which was secondary to decreased litter size and decreased fetal body weights (see body weight data from 0-18 GD in the Table below. The average maternal body weight gain was significantly reduced (p≤0.01) at the end of the dosing period compared to controls (81% of controls on GD 12 to 16). After correcting the maternal body weight for the gravid uterine weights (GD 18C = corrected body weight), the average body weight on GD 18 and the corrected maternal body weight gain was comparable between the dose groups.

Figure 20: Body Weight Data in Dams Treated with Venetoclax in the Embryo-Fetal Development Study with A-1195425 in Mice (Uncorrected Weights)

(Excerpted from Applicant's NDA)

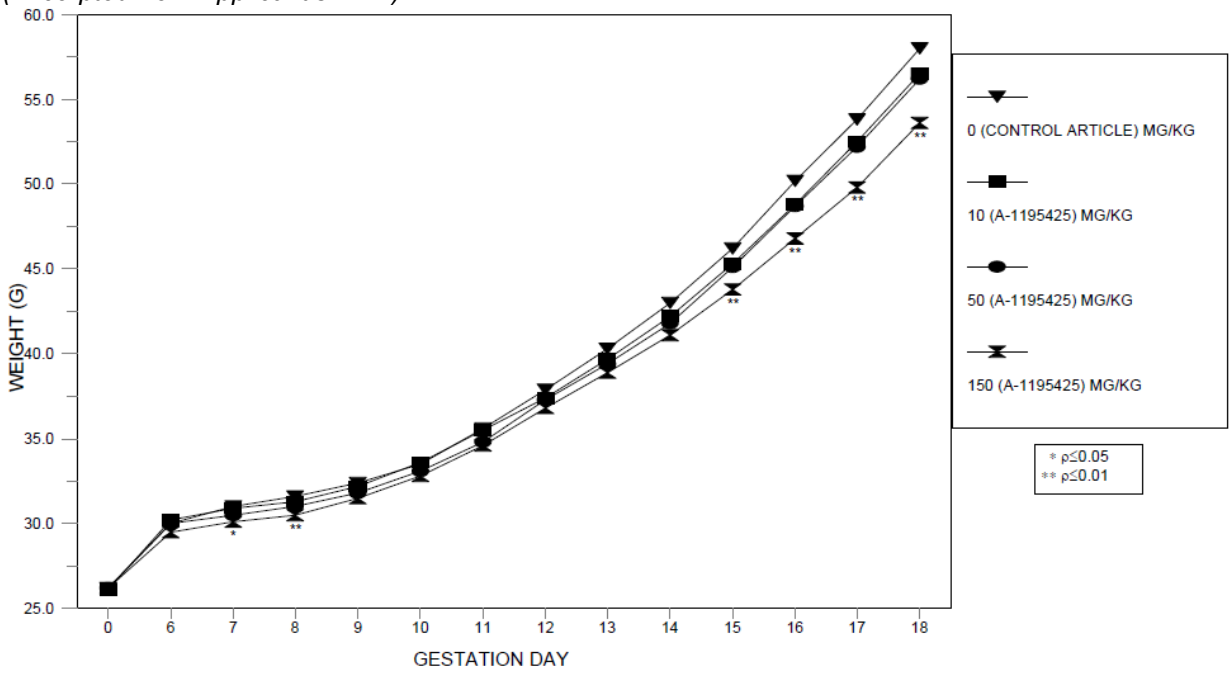


Table 53: Body Weight Changes in Dams Treated with Venetoclax in the Embryo-Fetal Development Study with Venetoclax in Mice

(Excerpted from Applicant's NDA)

TABLE 3 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - SUMMARY

GROUP TEST MATERIAL A-1195425 DOSE LEVEL (MG/KG)a		1 CONTROL ARTICLE 0	2 A-1195425 10	3 A-1195425 50	4 A-1195425 150
MICE TESTED	N	25	25	25	25
PREGNANT	N	24	25	25	25
MATERNAL BODY WEIGHT CHANGE (G)					
DAYS 0 - 6	MEAN±S.D.	+3.9 ± 0.8	+4.1 ± 0.8	+3.9 ± 1.0	+3.4 ± 0.8*
DAYS 6 - 9	MEAN±S.D.	+2.3 ± 0.6	+2.0 ± 0.7	+1.8 ± 0.6**	+2.0 ± 0.6
DAYS 9 - 12	MEAN±S.D.	+5.6 ± 0.8	+5.2 ± 1.4	+5.5 ± 1.0	+5.3 ± 1.0
DAYS 12 - 16	MEAN±S.D.	+12.2 ± 1.9	+11.4 ± 2.1	+11.4 ± 1.4	+9.9 ± 2.7**
DAYS 6 - 16	MEAN±S.D.	+20.1 ± 2.8	+18.6 ± 3.5	+18.6 ± 2.2	+17.2 ± 3.0**
DAYS 16 - 18	MEAN±S.D.	+8.0 ± 1.2 [22]b	+7.7 ± 1.6	+7.5 ± 1.1	+6.9 ± 1.8
DAYS 6 - 18	MEAN±S.D.	+28.1 ± 3.8 [22]b	+26.3 ± 4.9	+26.2 ± 3.0	+24.1 ± 4.7**
DAYS 0 - 18	MEAN±S.D.	+31.9 ± 4.3 [22]b	+30.4 ± 5.2	+30.0 ± 3.6	+27.4 ± 5.0**
DAYS 6 - 18C c	MEAN±S.D.	+5.9 ± 2.1 [22]b	+5.8 ± 1.7	+5.0 ± 1.2	+6.0 ± 1.4
DAYS 0 - 18C c	MEAN±S.D.	+9.7 ± 2.4 [22]b	+9.8 ± 1.8	+8.9 ± 1.6	+9.4 ± 1.8

DAYS = GESTATION DAYS
[ ] = NUMBER OF VALUES AVERAGED
a. Dose administration occurred on Gestation Days 6 through 15.
b. Excludes values for dams that were euthanized due to delivery.
c. 18C = Corrected maternal body weight (Gestation Day 18 body weight minus the gravid uterine weight).
* Significantly different from the control group value (p≤0.05).
** Significantly different from the control group value (p≤0.01).

Table 54: Summary of Embryonic Data at Scheduled Necropsy in Venetoclax Treated Females

(Excerpted from Applicant's NDA)

TABLE 5 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY

GROUP TEST MATERIAL A-1195425 DOSE LEVEL (MG/KG)a		1 CONTROL ARTICLE 0	2 A-1195425 10	3 A-1195425 50	4 A-1195425 150
LITTERS WITH ONE OR MORE LIVE FETUSES	N	22	25	25	24
IMPLANTATIONS	MEAN±S.D.	14.4 ± 1.7	13.3 ± 2.2	13.9 ± 1.6	13.4 ± 1.3
LIVE FETUSES	N	299	306	328	284
	MEAN±S.D.	13.6 ± 2.0	12.2 ± 2.2	13.1 ± 1.8	11.8 ± 2.9
LIVE MALE FETUSES	N	140	139	175	141
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	46.6 ± 13.9	45.2 ± 11.4	53.1 ± 14.5	52.6 ± 22.1
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	1.26 ± 0.11	1.27 ± 0.11	1.25 ± 0.08	1.19 ± 0.10*
MALE FETUSES	MEAN±S.D.	1.29 ± 0.11	1.30 ± 0.12	1.28 ± 0.08	1.21 ± 0.10*
FEMALE FETUSES	MEAN±S.D.	1.23 ± 0.11	1.25 ± 0.11	1.21 ± 0.09	1.18 ± 0.10 [23]b
% DEAD OR RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	6.0 ± 7.0	7.7 ± 6.9	5.5 ± 6.4	12.5 ± 20.2

[ ] = NUMBER OF VALUES AVERAGED
a. Dose administration occurred on Gestation Days 6 through 15.
b. Litter 2884 had no female fetuses.
* Significantly different from the control group value (p≤0.05).

Toxicokinetics

On GD 6 and 15 (or GD 7 and 16 for the 24-hour collection timepoint), blood was collected at approximately 1, 3, 6, 12 and 24 hours postdose. Following maternal blood collection on GD 15 or 16, fetal blood samples were collected.

- The mean maternal exposure (AUC) appeared to be approximately dose proportional in all three dose groups on GD 6.
- The mean maternal exposure on GD 15 was approximately dose proportional in the 10 and 50 mg/kg dose groups, and was less than dose proportional in the 150 mg/kg dose group.

Table 55: Mean Maternal Toxicokinetic Parameters for Venetoclax in the Embryo-Fetal Development Study

(Excerpted from Applicant's NDA)

	Gestation Day 6			Gestation Day 15		
Dose (mg/kg)	Cmax (µg/mL)	Tmax (hr)	AUC (µg*hr/mL)	Cmax (µg/mL)	Tmax (hr)	AUC (µg*hr/mL)
10	0.668	1.0	2.64	0.962	3.0	5.85
50	1.72	1.0	10.7	4.41	3.0	26.1
150	3.92	6.0	30.8	4.12	3.0	37.8

Table 56: Mean Fetal Toxicokinetic Parameters for Venetoclax in the Embryo-Fetal Development Study

(Excerpted from Applicant's NDA)

	Gestation Day 15				
Dose (mg/kg)	Fetal Cmax (µg/mL)	Fetal Tmax (hr)	Fetal AUC (µg*hr/mL)	Maternal AUC (µg*hr/mL)	AUC Ratio (Fetal/Maternal)
10	0.018	3.0	0.045	5.85	0.00769
50	0.209	12.0	3.24	26.1	0.124
150	0.342	12.0	5.35	37.8	0.142

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

- Venetoclax-related ovarian and uterine parameters were limited to 150 mg/kg when compared to controls.
- Increase in the average number of early resorptions per litter in the 150 mg/kg dose group was 1.9 resorptions/litter vs. 0.6 in controls.
- Increase in the percentage of postimplantation loss was 16.0% vs. 6.0% in controls.
- The percentage of dead or resorbed conceptuses per litter was 12.5% vs. 6.0% in controls).
- The average litter size and the average number of live fetuses per litter was statistically significant decrease ($p \leq 0.05$) in 150 mg/kg (11.4 vs. 13.6 in controls).

Table 57: Summary of Cesarean Section Data in Dams Treated with Venetoclax

(Excerpted from Applicant's NDA)

Parameter	Unit	0 mg/kg	10 mg/kg	50 mg/kg	150 mg/kg	Historical Control Data[a]
No. Pregnant	N (%)	24 (96.0%)	25 (100.0%)	25 (100.0%)	25 (100.0%)	90.6% (60.0-100.0%)
Litter Size	Mean ± SD	13.6 ± 2.0	12.3 ± 2.3	13.2 ± 1.7	11.4 ± 3.7*	Not tabulated in HCD
Live Fetuses/Litter	Mean ± SD	13.6 ± 2.0	12.2 ± 2.2	13.1 ± 1.8	11.4 ± 3.7*	12.4 (9.7-14.0)
Total Resorptions/Litter	Mean ± SD	0.8 ± 1.0	1.0 ± 0.9	0.7 ± 0.8	2.2 ± 3.5	1.0 (0.6-2.1)
Early Resorptions/Litter	Mean ± SD	0.6 ± 1.0	0.8 ± 0.8	0.5 ± 0.7	1.9 ± 3.2	0.8 (0.4-1.6)
% Postimplantation loss	Mean ± SD	6.0 ± 7.0	7.7 ± 6.9	5.5 ± 6.4	16.0 ± 26.4	7.7 (3.2-15.5)
Dams with all resorbed conceptuses	N(%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (4.0%)	0.2% (0.0-9.1%)
% Dead or Resorbed Conceptuses/Litter[b]	Mean ± SD	6.0 ± 7.0	7.7 ± 6.9	5.5 ± 6.4	12.5 ± 20.2	7.6% (5.3-10.4%)

[a] Testing Facility Historical Control Data compiled between June 2007 to June 2011 in mice based on 28 embryo-fetal developmental toxicity studies that evaluated 618 pregnant mice on GD 18.
[b] This value is based on dams with one or more live fetuses.
Underlined = A-1195425-related
* Significantly different from the control group value ($p \leq 0.05$).

Offspring (Malformations, Variations, etc.)

A statistically significant fetal body weights loss (6%) was observed in male and combined (male and female) weights at 150 mg/kg dose.

Table 58: Summary of A-1195425-Related Fetal Body Weights Changes

(Excerpted from Applicant's NDA)

Parameter	Unit	0 mg/kg	10 mg/kg	50 mg/kg	150 mg/kg	Historical Control Data[a]
Combined Fetal Body Weights (g)	Mean ± SD	1.26 ± 0.11	1.27 ± 0.11	1.25 ± 0.08	1.19 ± 0.10*	1.32 (1.20-1.37)
Male Fetal Body Weights (g)	Mean ± SD	1.29 ± 0.11	1.30 ± 0.12	1.28 ± 0.08	1.21 ± 0.10*	1.35 (1.22-1.40)
Female Fetal Body Weights (g)	Mean ± SD	1.23 ± 0.11	1.25 ± 0.11	1.21 ± 0.09	1.18 ± 0.10	1.29 (1.19-1.34)

[a] Testing Facility Historical Control Data compiled between June 2007 to June 2011 in mice based on 28 embryo-fetal developmental toxicity studies that evaluated 618 pregnant mice on GD 18.
Underlined = A-1195425-related
* Significantly different from the control group value ($p \leq 0.05$).

Fetal Examinations

There were no A-1195425-related fetal external, soft tissue or skeletal fetal abnormalities (malformations or variations) at any dose level.

Table 59: Malformations and Variations Observed in Litters from Dams Treated with Venetoclax

(Excerpted from Applicant's NDA)

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	A-1195425	A-1195425	A-1195425
A-1195425 DOSE LEVEL (MG/KG)a		0	10	50	150
LITTERS EVALUATED	N	22	25	25	24b
LITTERS WITH LIVE FETUS(ES)	N	22	25	25	24
FETUSES EVALUATED	N	300	308	329	284
LIVE	N	299	306	328	284
DEAD c	N	1	2	1	0
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	18(81.8)	21(84.0)	23(92.0)	22(91.7)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	61(20.4)	66(21.6)	69(21.0)	45(15.8)
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	20.6 ± 19.7	21.3 ± 16.2	20.8 ± 14.3	19.5 ± 19.7

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	A-1195425	A-1195425	A-1195425
A-1195425 DOSE LEVEL (MG/KG)a		0	10	50	150
LITTERS EVALUATED	N	22	25	25	24
LITTERS WITH LIVE FETUS(ES)	N	22	25	25	24
FETUSES EVALUATED	N	300	308	329	284
LIVE	N	299	306	328	284
DEAD b	N	1	2	1	0
FORE AND/OR HINDLIMBS: ROTATED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.3)	1(0.4)
PALATE: CLEFT					
LITTER INCIDENCE	N(%)	2(9.1)	1(4.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	2(0.7)	1(0.3)	0(0.0)	0(0.0)
HEAD: EXENCEPHALY					
LITTER INCIDENCE	N(%)	1(4.5)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.3)	0(0.0)	0(0.0)	0(0.0)
BODY: UMBILICAL HERNIA					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.3)	0(0.0)	0(0.0)
EYE LID(S): OPEN					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.0)	1(4.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.3)	1(0.3)	0(0.0)

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	A-1195425	A-1195425	A-1195425
A-1195425 DOSE LEVEL (MG/KG)a		0	10	50	150
LITTERS EVALUATED	N	22	25	25	23
LITTERS WITH LIVE FETUS(ES)	N	22	25	25	23
FETUSES EVALUATED	N	144	151	157	136
LIVE	N	143	150	157	136
DEAD b	N	1	1	0	0
PALATE: CLEFT					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.0)	1(4.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)	1(0.6)	0(0.0)
EYES: RETINA FOLDED					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.0)	1(4.0)	1(4.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)	1(0.6)	1(0.7)
EYES: LIDS OPEN					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.0)	1(4.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)	1(0.6)	0(0.0)
VESSELS: UMBILICAL ARTERY DESCENDS TO THE LEFT OF THE URINARY BLADDER					
LITTER INCIDENCE	N(%)	12(54.5)	9(36.0)	10(40.0)	11(47.8)
FETAL INCIDENCE	N(%)	17(11.9)	13(8.7)c	14(8.9)	12(8.8)
KIDNEYS: ABSENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.3)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)
INTESTINES: PROTRUDE THROUGH THE UMBILICUS					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)c	0(0.0)	0(0.0)

a. Dose administration occurred on Gestation Days 6 through 15.

Table 60: Malformations and Variations Observed in Litters from Dams Treated with Venetoclax (contd.)

(Excerpted from Applicant's NDA)

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	A-1195425	A-1195425	A-1195425
A-1195425 DOSE LEVEL (MG/KG)a		0	10	50	150
LITTERS EVALUATED	N	22	25	25	24
LITTERS WITH LIVE FETUS(ES)	N	22	25	25	24
FETUSES EVALUATED b	N	156	157	172	148
LIVE	N	156	156	171	148
DEAD c	N	0	1	1	0
THORACIC VERTEBRAE: CENTRA, FUSED					
LITTER INCIDENCE	N(%)	1(4.5)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
RIBS: THICKENED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)
RIBS: INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)
MANUBRIUM: IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	1(4.5)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
MANUBRIUM: FUSED					
LITTER INCIDENCE	N(%)	1(4.5)	1(4.0)	1(4.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	1(0.6)	1(0.6)	0(0.0)
STERNAL CENTRA: INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	1(4.5)	0(0.0)	1(4.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	1(0.6)	0(0.0)
STERNAL CENTRA: ASYMMETRIC					
LITTER INCIDENCE	N(%)	4(18.2)	0(0.0)	3(12.0)	2(8.3)
FETAL INCIDENCE	N(%)	4(2.6)	0(0.0)	4(2.3)	3(2.0)
STERNAL CENTRA: FUSED					
LITTER INCIDENCE	N(%)	1(4.5)	1(4.0)	1(4.0)	1(4.2)
FETAL INCIDENCE	N(%)	1(0.6)	1(0.6)	1(0.6)	1(0.7)

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	A-1195425	A-1195425	A-1195425
A-1195425 DOSE LEVEL (MG/KG)a		0	10	50	150
LITTERS EXAMINED	N	22	25	25	24
FETUSES EXAMINED	N	156	156	171	148
OSSIFICATION SITES PER FETUS PER LITTER					
HYOID	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	0.99 ± 0.04	0.98 ± 0.06
VERTEBRAE					
CERVICAL	MEAN±S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
THORACIC	MEAN±S.D.	13.38 ± 0.27	13.31 ± 0.41	13.23 ± 0.30	13.22 ± 0.27
LUMBAR	MEAN±S.D.	5.61 ± 0.26	5.64 ± 0.34	5.74 ± 0.29	5.74 ± 0.26
SACRAL	MEAN±S.D.	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
CAUDAL	MEAN±S.D.	7.34 ± 0.98	7.76 ± 1.26	7.77 ± 1.28	7.64 ± 0.98
RIBS (PAIRS)	MEAN±S.D.	13.31 ± 0.23	13.32 ± 0.30	13.21 ± 0.28	13.18 ± 0.23
STERNUM					
MANUBRIUM	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
STERNAL CENTERS	MEAN±S.D.	4.00 ± 0.05	4.00 ± 0.12	4.02 ± 0.09	4.01 ± 0.04
XIPHOID	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
FORELIMB b					
CARPALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
METACARPALS	MEAN±S.D.	4.00 ± 0.02	3.99 ± 0.04	4.00 ± 0.02	4.00 ± 0.00
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	11.33 ± 0.90	11.08 ± 1.51	11.16 ± 0.92	11.09 ± 0.73
HINDLIMB b					
TARSALS	MEAN±S.D.	0.58 ± 0.35	0.58 ± 0.36	0.57 ± 0.39	0.49 ± 0.41
METATARSALS	MEAN±S.D.	4.99 ± 0.05	4.98 ± 0.06	4.98 ± 0.05	5.00 ± 0.00
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	10.19 ± 1.00	10.05 ± 1.48	10.15 ± 1.16	10.23 ± 0.74

a. Dose administration occurred on Gestation Days 6 through 15.
b. Calculated as average per limb.

9.3 Embryonic Fetal Development

Study title: An Embryo-fetal Development Study of A-1195425 (b) (4) by Oral Gavage in Rabbits

Study no.:	R&D/12/824 (Sponsor Reference No. TE12-054)
Study report location:	4.2.3.5.2.
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	August 7, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1195425 (venetoclax, A- 195425.0 (b) (4), ABT-199 (b) (4) or A-1195425.0), 97059-1, 99.1%.

Key Study Findings:

- Four pregnant rabbits were euthanized or found dead at high dose 300 mg/kg/day.
- High dose 300 mg/kg/day showed reduction in body weight and food consumption.
- There were no effects on ovarian or uterine parameters, embryo-fetal survival, fetal body weights, and no fetal external, soft tissue or skeletal malformations or variations in the A-1195425 treated groups.
- The no-observed-adverse-effect level (NOAEL) for maternal toxicity was 100 mg/kg, based on mortality, clinical signs, maternal body weight loss and/or decreases in maternal body weight gain and decreases in maternal food consumption.
- The NOAEL for embryo-fetal development was 300 mg/kg, the highest dose tested.

Methods

Doses:	0, 50, 100, and 300 mg/kg
Frequency of dosing:	once daily on gestation days (GD) 7 through 19
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	(b) (4) suspended in Sterile Water for Injection, USP
Species/Strain:	New Zealand White [Hra:(NZW)SPF] female rabbits
Number/Sex/Group:	20
Satellite groups:	5 for TK
Study design:	See below

Experimental Design

Group No.	Test Material	A-1195425 Dose Level (mg/kg)	A-1195425 Concentration (mg/mL)[a]	Dose Volume (mL/kg)	No. of Main Study Rabbits (Assigned Rabbit Numbers)	No. of Toxicokinetic Rabbits (Assigned Rabbit Numbers)[b]
1	Control Article	0	0	10	20 (3601 -3620)	5 (3681 - 3685)
2	A-1195425	50	5	10	20 (3621 - 3640)	5 (3686 - 3690)
3	A-1195425	100	10	10	20 (3641 - 3660)	5 (3691 - 3695)
4	A-1195425	300	30	10	20 (3661 - 3680)	5 (3696 - 3700)

[a] The test article A-1195425, suspended in Sterile Water for Injection (SWFI), USP; assigned chemical potency = 118.9 mg A-1195425.0/gram (b) (4) formulation concentrations were adjusted for assigned chemical potency (correction factor = 8.41). The control article, Placebo (b) (4), was suspended in SWFI, USP, and administered in an amount equal to that given at the high dose; correction factor = 7.41.

[b] Toxicokinetic rabbits were used for blood collection purposes only.

Observations and Results

Mortality

At 300 mg/kg, four pregnant rabbits were found dead or euthanized between GD 16 and GD 25.

Table 61: Mortality in Rabbit Treated with Venetoclax in Embryo-Fetal Development Study

Dose	Animal #	GD Day	Cause
300 mg/kg	3661	found dead on GD 23	Abnormal fecal output, thin body condition, dehydration, decreased motor activity, impaired righting reflex, ptosis, cold to touch, bradypnea, red/purple sclera and reduced grooming; Loss of body weight and decreased food consumption; At necropsy, the serosal surface of the cecum and the mucosal surface in the cardiac region of the stomach were dark red and there were numerous black areas in the cardiac and fundic regions of the stomach. In addition, the walls were thickened in the pyloric region of the stomach and the mucosal surface in the duodenum had raised tan areas.
300 mg/kg	3665	euthanized on GD 16	Abnormal feces, decreased motor activity, dehydration, ptosis, hunched posture, thin body condition, bradypnea, pale mucous membranes; loss of body weight and decreased food consumption. At necropsy, the stomach and the intestines were distended with gas.
300 mg/kg	3666	found dead on GD 25	Abnormal feces, decreased motor activity, thin body condition, low carriage, dehydration, e cage pan; loss of body weight and decreased food consumption. At necropsy, the walls were thick in the pyloric region of the stomach.
300 mg/kg	3676	found dead on GD 24	Abnormal feces, mild dehydration, thin body condition, hunched posture, a swollen vulva; loss of body weight and decreased food consumption. At necropsy, all tissues appeared normal.

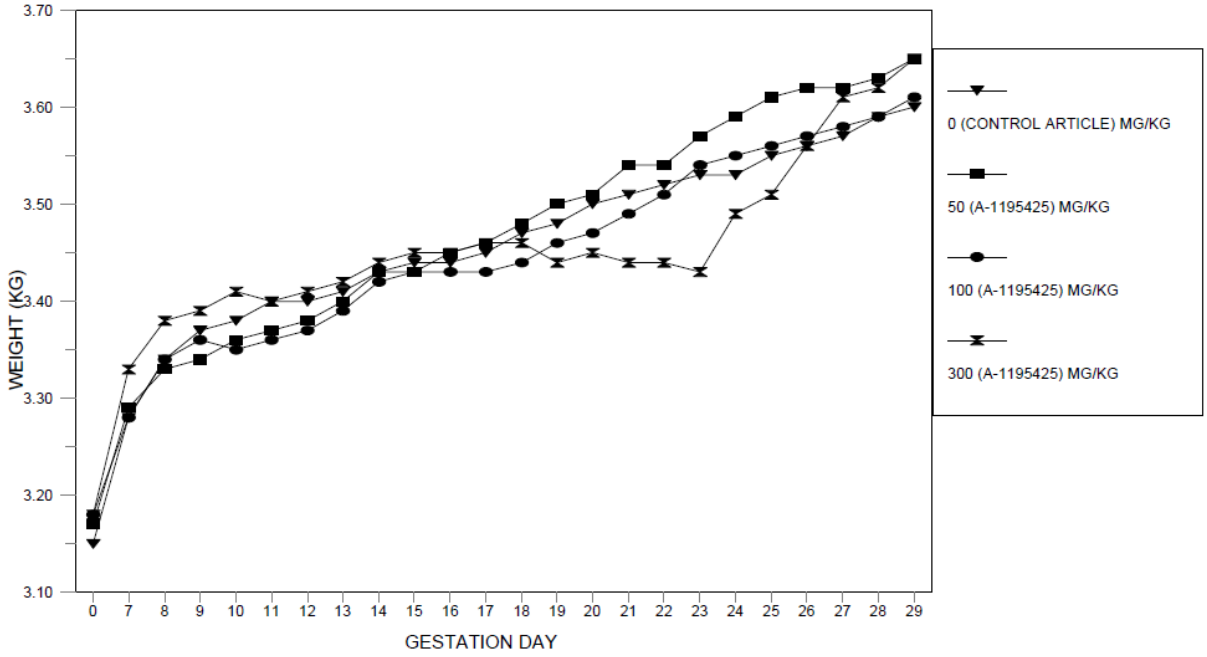
Clinical Signs

Scant feces, fecal stained fur, and thin body condition at 300 mg/kg.

Body Weight
In the 300 mg/kg dose group, there was a statistically significant loss (p≤0.01; -0.01 kg) in maternal body weight at the end of the dose period (GD 16 to 20) compared to a body weight gain of +0.05 kg in controls.

Figure 21: Maternal Body Weights in Embryo Fetal Development Study in Rabbits

(Excerpted from Applicant's NDA)



Food Consumption
A statistically significant reduction in maternal food consumption (g/day and g/kg/day) was observed (p≤0.01) on GD 16 to 20 compared to controls. Absolute and relative food consumption was 79% and 78% of controls, respectively, on GD 7 to 20.

Toxicokinetics
The mean exposure (AUC) appeared to be less than proportional to dose at all three dose levels on GD 7 and GD 19. The mean estimates of the venetoclax toxicokinetic parameters are summarized in Table 70 and Table 71.

Table 62: Maternal Toxicokinetic Parameters in Embryo-Fetal Development Study in Rabbits on Gestation Day 7 and 19

(Excerpted from Applicant's NDA)

Dose (mg/kg)	Gestation Day 7			Gestation Day 19		
	Cmax (ng/mL)	Tmax (hr)	AUC (ng*hr/mL)	Cmax (ng/mL)	Tmax (hr)	AUC (ng*hr/mL)
50	339 ± 127	3.6 ± 1.3	2290 ± 1020	226 ± 53.4	3.0 ± 0.0	1380 ± 428
100	424 ± 95.0	2.6 ± 0.9	2910 ± 485	318 ± 154	2.6 ± 0.9	2370 ± 1230
300	702 ± 157	4.2 ± 1.6	6120 ± 2040	416 ± 121	2.2 ± 1.1	4900 ± 1300

Table 63: Fetal Toxicokinetic Parameters in Embryo-fetal development Study in Rabbits

Dose (mg/kg)	Fetal Concentration (ng/mL)	Maternal Concentration (ng/mL)	Ratio (Fetal:Maternal)
50	3.21 ± 3.16	136 ± 44.0	0.0243 ± 0.0262
100	9.10 ± 4.03	217 ± 108	0.0441 ± 0.0107
300	17.2 ± 4.07	242 ± 102	0.0759 ± 0.0163

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)
Unremarkable

Ovarian and Uterine Examinations

Table 64: Caesarean Observations in Embryo-Fetal Development Study in Rabbits

(Excerpted from Applicant's NDA)

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	A-1195425	A-1195425	A-1195425
DOSE LEVEL (MG/KG)a		0	50	100	300
RABBITS TESTED	N	20	20	20	20
PREGNANT	N(%)	19(95.0)	19(95.0)	18(90.0)	20(100.0)
FOUND DEAD	N(%)	0(0.0)	0(0.0)	0(0.0)	3(15.0)**b-d
UNSCHEDULED EUTHANASIA	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)e
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON GESTATION DAY 29	N	19	19	18	16
CORPORA LUTEA	MEAN±S.D.	9.1 ± 1.9	9.5 ± 1.4	9.7 ± 2.2	9.7 ± 1.4
IMPLANTATIONS	MEAN±S.D.	8.1 ± 2.3	8.6 ± 1.4	8.9 ± 2.4	8.8 ± 1.7
% PREIMPLANTATION LOSS	MEAN±S.D.	11.5 ± 17.0	8.8 ± 6.0	8.4 ± 7.8	9.8 ± 9.2
LITTER SIZES	MEAN±S.D.	8.0 ± 2.3	8.4 ± 1.5	8.5 ± 1.8	8.5 ± 1.6
LIVE FETUSES	N	152	159	153	136
	MEAN±S.D.	8.0 ± 2.3	8.4 ± 1.5	8.5 ± 1.8	8.5 ± 1.6
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.1 ± 0.3	0.3 ± 0.7	0.4 ± 1.0	0.2 ± 0.4
EARLY RESORPTIONS	N	2	3	4	3
	MEAN±S.D.	0.1 ± 0.3	0.2 ± 0.7	0.2 ± 0.5	0.2 ± 0.4
LATE RESORPTIONS	N	0	2	4	1
	MEAN±S.D.	0.0 ± 0.0	0.1 ± 0.3	0.2 ± 0.9	0.1 ± 0.2
% POSTIMPLANTATION LOSS	MEAN±S.D.	1.3 ± 4.0	3.0 ± 8.9	3.8 ± 7.8	2.6 ± 4.7

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE FETUSES) / NUMBER OF IMPLANTATIONS] x 100
a. Dose administration occurred on Gestation Days 7 through 19.
b. Rabbit 3661 was found dead on Gestation Day 23.
c. Rabbit 3666 was found dead on Gestation Day 25.
d. Rabbit 3676 was found dead on Gestation Day 24.
e. Rabbit 3665 was euthanized on Gestation Day 16 due to adverse clinical observations.
** Significantly different from the control group value (p≤0.01).

Offspring (Malformations, Variations, etc.)

Fetal Body Weights

Unremarkable

Fetal Examinations

Unremarkable

Table 65: Fetal Alterations in Embryo Fetal Development Study in Rabbits

(Excerpted from Applicant's NDA)

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	A-1195425	A-1195425	A-1195425
DOSE LEVEL (MG/KG)a		0	50	100	300
LITTERS EVALUATED	N	19	19	18	16
LITTERS WITH LIVE FETUS(ES)	N	19	19	18	16
FETUSES EVALUATED	N	152	159	153	136
LIVE	N	152	159	153	136
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	6(31.6)	8(42.1)	10(55.6)	5(31.2)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	6(3.9)	10(6.3)	18(11.8)**	6(4.4)
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	3.7 ± 6.1	6.0 ± 7.9	11.9 ± 13.0	4.2 ± 6.8

a. Dose administration occurred on Gestation Days 7 through 19.
** Significantly different from the control group value (p≤0.01).

Table 66: Fetal Gross External Alterations - Caesarean-Delivered Live Fetuses

(Excerpted from Applicant's NDA)

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	A-1195425	A-1195425	A-1195425
DOSE LEVEL (MG/KG)a		0	50	100	300
LITTERS EVALUATED	N	19	19	18	16
LITTERS WITH LIVE FETUS(ES)	N	19	19	18	16
FETUSES EVALUATED	N	152	159	153	136
LIVE	N	152	159	153	136
HEAD: MENINGOCELE					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.6)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)	0(0.0)
FORE AND/OR HINDLIMBS ROTATED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.6)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)	0(0.0)
TAIL: ABSENT					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)	0(0.0)	0(0.0)

a. Dose administration occurred on Gestation Days 7 through 19.

Table 67: Fetal Soft Tissue Alterations - Caesarean-Delivered Live Fetuses

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	A-1195425	A-1195425	A-1195425
DOSE LEVEL (MG/KG)a		0	50	100	300
LITTERS EVALUATED	N	19	19	18	16
LITTERS WITH LIVE FETUS(ES)	N	19	19	18	16
FETUSES EVALUATED	N	152	159	153	136
LIVE	N	152	159	153	136
BRAIN: MODERATE VENTRICULAR DILATION					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)	0(0.0)	0(0.0)
HEART: INTERVENTRICULAR SEPTAL DEFECT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.6)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)d	0(0.0)
VESSELS: RIGHT SUBCLAVIAN ARISES TO LEFT OF THE LEFT SUBCLAVIAN					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.6)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)d	0(0.0)
VESSELS: INNOMINATE ARTERY ABSENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.6)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)d	0(0.0)
VESSELS: RIGHT SUBCLAVIAN PASSES DORSAL TO TRACHEA AND ESOPHAGUS					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.6)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)d	0(0.0)
LIVER: THICK					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.6)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	2(1.3)b,c	0(0.0)
LIVER: PALE					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.6)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	2(1.3)b,c	0(0.0)
GALLBLADDER: ABSENT					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)	0(0.0)	0(0.0)

a. Dose administration occurred on Gestation Days 7 through 19.
b. Fetus 3650-4 had other soft tissue alterations.
c. Fetus 3650-11 had other soft tissue alterations.
d. Fetus 3658-5 had other soft tissue alterations.

Table 68: Fetal Skeletal Alterations - Caesarean-Delivered Live Fetuses

(Excerpted from Applicant's NDA)

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	A-1195425	A-1195425	A-1195425
DOSE LEVEL (MG/KG)a		0	50	100	300
LITTERS EVALUATED	N	19	19	18	16
LITTERS WITH LIVE FETUS(ES)	N	19	19	18	16
FETUSES EVALUATED	N	152	159	153	136
LIVE	N	152	159	153	136
RIBS: EXTRA					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.6)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)f	0(0.0)
MANUBRIUM: IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.6)	1(6.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)e	1(0.7)
STERNAL CENTRA: FUSED					
LITTER INCIDENCE	N(%)	2(10.5)	0(0.0)	3(16.7)	1(6.2)
FETAL INCIDENCE	N(%)	2(1.3)b	0(0.0)	3(2.0)e	1(0.7)
STERNAL CENTRA: ASYMMETRIC					
LITTER INCIDENCE	N(%)	1(5.3)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)b	1(0.6)	0(0.0)	0(0.0)
PELVIS: PUBIS, NOT OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.6)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	2(1.3)	0(0.0)

a. Dose administration occurred on Gestation Days 7 through 19.
b. Fetus 3614-4 had other skeletal alterations.
c. Fetus 3631-4 had other skeletal alterations.
d. Fetus 3647-5 had other skeletal alterations.
e. Fetus 3658-5 had other skeletal alterations.
f. Fetus 3660-7 had other skeletal alterations.
g. Fetus 3660-9 had other skeletal alterations.

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	A-1195425	A-1195425	A-1195425
DOSE LEVEL (MG/KG)a		0	50	100	300
LITTERS EVALUATED	N	19	19	18	16
LITTERS WITH LIVE FETUS(ES)	N	19	19	18	16
FETUSES EVALUATED	N	152	159	153	136
LIVE	N	152	159	153	136
HYOID: ALA, ANGULATED					
LITTER INCIDENCE	N(%)	2(10.5)	3(15.8)	1(5.6)	1(6.2)
FETAL INCIDENCE	N(%)	2(1.3)	3(1.9)	2(1.3)	2(1.5)
THORACIC VERTEBRAE: HEMIVERTEBRA					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)c	0(0.0)	0(0.0)
THORACIC VERTEBRAE: CENTRUM, UNILATERAL OSSIFICATION					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.6)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)g	0(0.0)
CAUDAL VERTEBRAE: MISALIGNED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.6)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	2(1.3)	0(0.0)
CAUDAL VERTEBRAE: 4 PRESENT					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)c	0(0.0)	0(0.0)
CAUDAL VERTEBRAE: ARCHES, FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)c	0(0.0)	0(0.0)
RIBS: THICKENED					
LITTER INCIDENCE	N(%)	1(5.3)	0(0.0)	1(5.6)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	1(0.6)	0(0.0)
RIBS: SPLIT					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	1(5.6)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)c	1(0.6)f	0(0.0)
RIBS: PROXIMATE					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.6)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)g	0(0.0)

Table 69: Fetal Ossification Sites - Caesarean-Delivered Live Fetuses

(Excerpted from Applicant's NDA)

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	A-1195425	A-1195425	A-1195425
DOSE LEVEL (MG/KG) a		0	50	100	300
LITTERS EXAMINED	N	19	19	18	16
FETUSES EXAMINED	N	152	159	153	136
OSSIFICATION SITES PER FETUS PER LITTER					
HYOID	MEAN±S.D.	0.99 ± 0.03	1.00 ± 0.00	0.98 ± 0.07	1.00 ± 0.00
VERTEBRAE					
CERVICAL	MEAN±S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
THORACIC	MEAN±S.D.	12.58 ± 0.31	12.55 ± 0.30	12.44 ± 0.28	12.59 ± 0.28
LUMBAR	MEAN±S.D.	6.41 ± 0.31	6.45 ± 0.30	6.55 ± 0.28	6.40 ± 0.26
SACRAL	MEAN±S.D.	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
CAUDAL	MEAN±S.D.	16.72 ± 0.34	16.81 ± 0.28	16.54 ± 0.35	16.71 ± 0.33
RIBS (PAIRS)	MEAN±S.D.	12.48 ± 0.30	12.49 ± 0.30	12.38 ± 0.26	12.55 ± 0.26
STERNUM					
MANUBRIUM	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
STERNAL CENTERS	MEAN±S.D.	3.87 ± 0.20	3.94 ± 0.11	3.90 ± 0.14	3.91 ± 0.12
XIPHOID	MEAN±S.D.	0.94 ± 0.11	0.99 ± 0.04	0.93 ± 0.16	0.98 ± 0.04
FORELIMB b					
CARPALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
METACARPALS	MEAN±S.D.	4.98 ± 0.05	4.98 ± 0.05	4.94 ± 0.17	4.98 ± 0.05
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	13.88 ± 0.19	13.90 ± 0.15	13.86 ± 0.19	13.94 ± 0.11
HINDLIMB b					
TARSALS	MEAN±S.D.	2.00 ± 0.00	2.00 ± 0.00	1.99 ± 0.05	2.00 ± 0.00
METATARSALS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
DIGITS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
PHALANGES	MEAN±S.D.	12.00 ± 0.00	12.00 ± 0.00	11.99 ± 0.05	12.00 ± 0.00

a. Dose administration occurred on Gestation Days 7 through 19.
b. Calculated as average per limb.

10. Special Toxicology Studies.

Study title: Multiple Dose Oral Phototoxicity Evaluation of A-1195425 (b) (4) in Hairless Female Mice

Study no.:	R&D/14/1173 (Sponsor Reference No. TD14-230)
Study report location:	4.2.3.7.7.
Conducting laboratory and location:	(b) (4) (b) (4)
Date of study initiation:	September 2, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1195425 (venetoclax, A- 195425.0 (b) (4), ABT-199 (b) (4) or A-1195425.0), 97059-1, 99.1%.

Key Findings:

- Venetpclax was not phototoxic in hairless female mice following UVR exposure.

Methods:

Strains :	Crl:SKH1-hr hairless mice
Concentrations in definitive study:	0, 200, and 825 mg/kg once daily for 3

	days
Basis of concentration selection:	The 825 mg/kg/day ~ maximum feasible dose in a previous in vivo mouse micronucleus study (AbbVie R&D/12/675) and was expected to give exposure close to expected efficacious human exposures.
Negative control:	0.5% methylcellulose (MC) in reverse osmosis (R.O.) processed deionized water
Positive control:	Sparfloxacin, 100 mg/kg/day
Formulation/Vehicle:	0.5% methylcellulose (MC) in reverse osmosis (R.O.) processed deionized water

Experimental Design

Group	No. of Mice per Group	Test Material	Dose Level (mg/kg/day)	Concentration (mg/mL)	Dose Volume[a]	Interval Between Formulation Administration and UVR Exposure[b] (Hours ± Minutes)	Assigned Mouse Numbers
1	5	Control Article	0	0	20	3 ± 30	1151-1155
2	10	A-1195425	200	10	20	3 ± 30	1156-1165
3	10	A-1195425	825	41.25	20	3 ± 30	1166-1175
4	5	Sparfloxacin	100	10	10	1 ± 10	1176-1180

[a] Dose volume for A-1195425 formulations was 20 mL/kg; for Sparfloxacin, 10 mL/kg.
[b] The interval between formulation administration and UVR exposure was based on the average dosing time for each group.

Study Design:
The test article and control formulations were administered once daily for three days by oral gavage to Crl:SKH1-hr hairless mice. Before UVR exposure, all mice were lightly anesthetized via intraperitoneal injection of chloral hydrate in deionized water. The mice were placed 0.87 meters from the UVR source at the time of exposure. A Solar Light™ detector was used to monitor the incident UVR, and a dose of 10.3 to 11.0 J/cm^2 of UVA and 145 to 155 mJ/cm^2 of UVB was delivered to each mouse over a period of 42 to 45 minutes.

In-life procedures including, clinical observations, dermal scoring, body weights and terminal sacrifice were performed. Terminal euthanasia was performed on Day 6.

Results:
- All mice survived until scheduled euthanasia.
- No adverse clinical observations were recorded for any mouse on study.

- There was no evidence of cutaneous phototoxicity, elicited by a single exposure to UVR, approximately 3 hours after the third and final consecutive daily oral administrations of A-1195425, up to 825 mg/kg/day.

Table 70: Summary of Skin Reaction and Clinical Observations in Multiple Dose Oral Phototoxicity Evaluation of A-1195425 in Hairless Female Mice

GROUP	1	2	3	4
TEST MATERIAL	CONTROL ARTICLE	A-1195425	A-1195425	SPARFLOXACIN
DOSE LEVEL (MG/KG)a	0	200	825	100
MICE TESTED	5	10	10	5
MORTALITY	0	0	0	0
SKIN REACTION OBSERVATIONS:				
SITE 1:				
ERYTHEMA: GRADE 1	0/ 0	0/ 0	0/ 0	20/ 5
EDEMA: GRADE 1	0/ 0	0/ 0	0/ 0	6/ 4
GRADE 2	0/ 0	0/ 0	0/ 0	4/ 3
FLAKING: GRADE 1	0/ 0	0/ 0	0/ 0	8/ 4
WRINKLING	0/ 0	0/ 0	0/ 0	1/ 1
CLINICAL OBSERVATIONS:				
		NO ADVERSE FINDINGS		

N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF MICE WITH OBSERVATION
a. For Groups 1 through 3, formulation administration occurred on Days 1 through 3 of study and UVR exposure occurred on Day 3 of study. For Group 4, formulation administration and UVR exposure occurred on Day 3 of study.

11 Integrated Summary and Safety Evaluation

Nonclinical pharmacology studies demonstrated that venetoclax is an inhibitor of the Bcl-2 anti-apoptotic protein. In a series of biochemical assays to characterize binding affinity, venetoclax demonstrated selectivity to Bcl-2 (Ki <0.1 nM) relative to both anti-apoptotic and pro-apoptotic complexes. Venetoclax killed FL5.12-Bcl-2 cells (cells engineered to be dependent on either Bcl-2 or Bcl-XL for survival upon interleukin-3 (IL-3) withdrawal) (EC_{50} = 4 nM), and showed much weaker activity against FL5.12-Bcl-XL cells (EC_{50} = 261 nM), indicating that this compound is functionally selective for Bcl-2. Venetoclax was effective in disrupting proapoptic Bcl-2-Bim complexes (EC_{50} = 3 nM), but was much less effective against Bcl-XL-Bcl-XS (EC_{50} = 2.167 µM) or Mcl-1-Noxa (EC_{50} >3.0 µM) complexes, further demonstrating the selectivity of this compound. Cell killing by venetoclax requires the pro-apoptotic family members Bax and/or Bak as demonstrated in Bak-/- Bax-/- double knockout murine embryonic fibroblasts (MEFs).

Venetoclax demonstrated cell killing activity against a variety of lymphoma and leukemia cells in vitro including FL, MCL, DLBCL, AML, and ALL and additionally in CLL cells derived from patients ex vivo. Venetoclax promotes cell death in a variety of hematological tumor cell lines including CLL cells derived from patients with an average EC50 of 6 nM (n=35). Venetoclax induced cell death in CLL samples bearing the 17p deletion derived from patients, with an average EC_{50} of 8 nM (n = 5). Additionally, In SCID mouse models of human xenografts expressing high levels of Bcl-2, treatment with venetoclax resulted in reduction in tumor volume. Venetoclax had high activity

against cell lines expressing high levels of Bcl-2. Venetoclax showed increased activity in Bcl-2 expressing tumor cells relative to other anti-apoptotic family members (Bcl-XL expressing cells).

Venetoclax was assessed for its ability to induce the intrinsic apoptotic program. A concentration dependent increase in the cytochrome C, caspase activation, and detection of phosphatidylserine expression with annexin V was observed in RS4;11 cells (human acute leukemia cell line with the t(4;11) chromosomal rearrangement). Venetoclax induced apoptotic cell death by release of mitochondrial intermembrane proteins cytochrome C, caspase activation which was followed by the externalization of phosphatidylserine at the cell membrane.

Venetoclax was assessed for its off-target activity across a panel of G-protein coupled receptors and voltage-gated ion channel binding sites. Venetoclax produced significant inhibition (> 50% inhibition on receptor binding) for prostacyclin (IP)(Ki 0.81 μM), peripheral benzodiazepine (BZD),(Ki 0.38 μM), and serotonin-5a (5-HT5a) receptors (0.37 μM) receptors. The affinity of venetoclax to Bcl-2 was 37,000-fold greater than the most off-target receptors. Venetoclax did not induce CNS effects in safety pharmacology studies in mice suggesting that the risk for off-target effects caused are minimal. M27, a major human metabolite of venetoclax produced >50% displacement of control-specific binding for DOP receptors (Ki 0.65 μM, IC_{50} 1.1 μM). M27 did not induce >50% agonist or antagonist activity at the DOP receptor up to a maximum concentration of 10 μM in a functional assay (R&D150007).

Safety pharmacology studies with venetoclax consisted of an in-vitro evaluation of hERG (human ether-á-go-go-related gene) channel interaction, a functional observational battery (FOB) for neurological and respiratory assessment in mice, and a cardiovascular safety pharmacology study in dogs. No venetoclax-induced adverse effects were seen in any of these studies. Venetoclax was a weak hERG blocker with a low potential to induce QT prolongation.

Tissue distribution was extensive in rats following administration of oral venetoclax. The highest exposure (by C_{max} or AUC) was in the liver, lymph nodes, small intestine, adrenal glands, kidney cortex, kidney, and the pancreas. Concentrations of radioactivity in eye lens and the non-circumventricular central nervous system (CNS) tissues (cerebellum, cerebrum, medulla, olfactory lobe, and spinal cord) in male and female rats, as well as bone and eye in the male rats were below measurable levels throughout the course of this study. Venetoclax metabolized following oral exposure in both mice and dogs; the metabolites in both mouse and dog plasma account for less than 13% of total drug related materials. “M27” was detected in both species (0.21% in dogs, 0.79% in mouse). The main route of elimination is the hepatobiliary. In both the mouse and the dog, approximately 90% of elimination occurred in the feces, and less than 1% in the urine. Based on the data collected in general toxicology studies, there were no gender differences in exposure, and increased in Cmax and AUC values were dose proportional. The elimination half-life after oral dosing in nonclinical species ranged from 3 to 14.5 hours.

The toxicity of repeated daily doses of oral venetoclax was assessed by conducting 26-week (6-month) and 39-week (9-month) toxicity studies in mice and dogs, respectively. In both mice and dogs, the major target organs of venetoclax toxicity included the lymphatic system, and male reproductive organs (dogs). Toxicities in mice and/or dogs included, dose-related body weight reductions (up to 15%) correlated with decreases in food consumption in dogs, decreases in lymphocyte (up to -75% in mice, and -81% in dogs) and red blood cell mass decreases in mice and dogs. Decrease in lymphocytes correlated with microscopic findings in lymphoid organs including the mandibular and mesenteric lymph nodes, and spleen, and gut-associated lymphoid tissue (GALT). Decreased prostate weights, dose dependent bilateral testicular seminiferous tubule degeneration/atrophy, reduced testicular weight in dogs, epithelial single cell necrosis (gallbladder, exocrine pancreas, prostate, epididymides, and stomach) in dogs and hair discoloration correlated microscopically with decreased pigment in the hair follicle bulbs in dogs.

Venetoclax was not phototoxic to Crl:SKH1-hr hairless female mice when administered orally daily for three days up to 825 mg/kg followed by UV exposure.

Venetoclax did not induce mutations in the bacterial mutagenesis (Ames) assay and, was not clastogenic in the in vitro chromosome aberration assay using human peripheral blood lymphocytes and in vivo bone marrow mouse micronucleus assay. Carcinogenicity studies have not been conducted and are not necessary for the proposed indications. M27 did not induce mutations in the bacterial mutagenesis (Ames) and in vitro chromosome aberration studies.

The effect of venetoclax on fertility and early development was evaluated in mice. In these studies venetoclax-treated male mice were mated to untreated female rats and venetoclax-treated female mice were mated to untreated male rats at 50-600 mg/kg by oral gavage. Male mice were administered with test or control article 14 days prior to cohabitation, during cohabitation and continuing through the day before euthanasia. Female mice were administered with test or control article once daily beginning 15 days before cohabitation, during cohabitation and continuing until gestation day (GD) 7. In fertility and early embryonic development studies conducted in male and female mice, venetoclax had no effect on male fertility, or female fertility parameters (e.g. estrous cycling, mating, or early embryonic development).

The embryo-fetal development effects of venetoclax were studied in mice and rabbits. Venetoclax produced decreases in implantations, litter size, live fetuses, fetal body weights, increases in both dead or resorbed conceptuses/litter and number of post implantation losses in mice. These effects are supported by scientific literature indicating the role of BCL-2 in oocyte and embryonic development. In addition, BCL-2 knockout mice exhibited adverse developmental effects, such as renal failure. Thus, administration of venetoclax during pregnancy may cause embryo-fetal toxicities and a statement under the Warnings and Precautions of the label is warranted. Venetoclax was not teratogenic in mice. Of note, the human metabolite M27, present at nearly 30%

in patients at the recommended dose of 400 mg/day was present in minor amounts in the mouse (0.8%). In rabbits, venetoclax was maternally toxic based on mortality (4/20) and reductions in net body weight gain (57% of the control), most evident at the high dose. While no embryo-fetal effects were observed in rabbits, this species may not predict adverse embryo-fetal effects in humans because the exposure to the parent drug was very low (0.2 times the human exposure) and metabolite M27 is not present in rabbits.

In conclusion, the Applicant conducted adequate nonclinical studies to support the use of venetoclax in patients with relapsed or refractory chronic lymphocytic leukemia (CLL) who have received at least one prior therapy, including those with 17p deletion. From a Pharmacology/Toxicology perspective, approval for venetoclax is recommended.

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/s/

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03/21/2016

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